ALMA MATER STUDIORUM – UNIVERSITÀ DI BOLOGNA

***DOTTORATO DI RICERCA IN SCIENZE E TECNOLOGIE AGRARIE, AMBIENTALI E ALIMENTARI***

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CICLO: **XXVI**

**Settore Concorsuale di afferenza: 07/E1 – Chimica Agraria, Genetica Agraria e Pedologia**

**Settore Scientifico disciplinare: AGR14/Pedologia**

***TITOLO TESI:***

# *Physical land degradation and loss of soil fertility: soil structural stability and bio-physical indicators*

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**Esame finale anno: 2014**

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**Acknowledgements**

A major research project like this is never the work of anyone alone. The contributions of many different people, in their different ways, have made this possible. So clearly, it would not have been possible to write this Ph.D thesis without the help and support of the kind people around me, to only some of whom it is possible to give particular mention here.

Above all, I would like to thank my wife Elisabeth for her personal support and great patience at all times. I would like also to thank her parents for their hospitality and for all sacrifices done for me. My parents, brothers and sisters have given me their unequivocal support throughout, as always, for which my mere expression of thanks likewise does not suffice. A big thank you to my uncle Dr. G. Samuel YABRE who strongly supported me during the preparation of my thesis. I would like to express my deep gratitude and respect to Carla FORCELLINI, Dr. Sergio GIORDANO and their family Guido, Franco, Andrea, Sara … for their great support. From them, I got all: their affection, financial support, encouragement, accompaniment at all steps of my adventure for doctoral studies.

This thesis would not have been possible without the help, support and patience of my principal supervisor. The good advice, support and friendship of my second supervisor, Prof. Gilmo VIANELLO, has been invaluable on both an academic and a personal level, for which I am extremely grateful. I would like to acknowledge all team members of Agricultural Chemistry laboratory, main laboratory that hosted me for my thesis works. Especially, I would like to express all my gratitude to Prof. Claudio CIAVATTA and his colleagues Profs. Ornella FRANCIOSO, Claudio MARZADORI, Livia Vittori ANTISARI affectionately called Titi, Dr. Luciano CAVANI, Dr. Andrea il Capo, Dr. Illaria, Dr. Paola, Dr. Daniella and Dr. Marco. They gave me their great support and attention. I remember the generosity and encouragement of Prof. Lorenzo BARBANTI and my colleagues Serena, Luigi, Caterina, Elena, Francesca, Enrico, Sonia etc during the “pranzo”.

I’m very grateful to Prof. Carmelo DAZZI, Prof. Andrea SEGRE, Dr. Clare WILSON for providing to me some important documents about Soil Science.

I also thank the Department of Agricultural Science for their support and assistance since the start of my previous Ph.D work, especially the head of department, Prof. Alberto VICARI and Prof. Giovanni DINELLI, the head of my Ph.D program.

My sincere thanks also goes to Prof. Achille FRANCHINI head of the former Food Science Department and to Prof. Luigi Filippo D’Antuono my first Tutor for their support which allowed me to do my first steps in Italy. I thank a lot Prof. Ivano DIONIGI, Magnificent Rector of ALMA MATER STUDIORUM for the support and collaboration of different structures of the Institution.

I can not forget to express my sincere thanks to the municipal authorities of Nivelles in Belgium, especially to Mr. Pierre HUART, Mayor for the help and support of Nivelles when I was in difficult time.

A lot of thanks to my former Profs. Edmond HIEN, Victor HIEN, Ablassé BILGO, Prosper ZOMBRE, Abdoulaye MANDO, Issaka Joseph BOUSSIM, Bernard BACYE and Mipro HIEN from Burkina Faso for their great support and encouragement.

Finally, I thank the religious authorities of Italy and Burkina Faso for their support. A big thank you to all those who said yes to me and to all those who said no.

# Chapter I: General introduction and review of literature

## 1. Motivation of the thesis and it context

Soil is a valuable natural resource that performs crucial ecosystem functions, and provides many valuable environmental goods and services like food, fiber and fuel production, carbon sequestration, water regulation and habitat provision (Costanza et al., 1997; Swinton et al., 2007). Changing climate will affect these functions and may also have serious consequences for the well being of people, who are dependent on the broad range of environmental goods and services regulated by soil (IPCC, 2007). The EU's Thematic Strategy for Soil Protection (EC, 2006) has also stated that several soil functions are under serious pressure in many parts of Europe. Soil is then one of the key life-support systems on our planet, responsible for major ecological and other functions such as what we have already mentioned above and for these reasons soil requires to us a great attention.

According to the different predictions models, climate change will lead to increasing surface temperatures worldwide and the European Alps had experienced a 2°C increase in annual minimum temperatures during the 20$^{th}$ century, with a marked rise since the early 1980s (Beniston et al., 1997). By the end of the current 21$^{st}$ century, global mean temperature is predicted to increase by 2–7 °C, and the amount and distribution of precipitation is predicted to change, due to increases in atmospheric greenhouse gas concentrations, especially carbon dioxide (IPCC, 2007; Wu et al., 2011). Global climate change with the possible increase of precipitations would significantly alter vegetation patterns and would then affect soil formation processes and its functions on the mountain ecosystems that are especially vulnerable (Schröter et al., 2005). Among the different threats to soil resources in Europe as the consequences of the global warming, erosion is a major one and may impair it ability to deliver the range of ecosystem goods and services. Physical pathways of soil loss predominate and fall within the domain of soil erosion, which is defined as the wearing away of the land surface by physical forces such as rainfall, flowing water, wind, ice, temperature change, gravity or other natural or anthropogenic agents that abrade, detach and remove soil or geological material from one point on the earth's surface to be deposited elsewhere.

In European's mountain environments, soil organic matter (SOM) is particularly important for ecosystem services and changes in SOM stocks and characteristics can affect the water and nutrient supply of forests and the stability of slopes, which may have dramatic socio-economic

consequences for mountain regions (IPCC, 2007). Soil organic materials are important soil additives to improve soil physical properties such as soil structure, of which degradation occurs mostly due to the decrease of soil organic matter often caused by excessive soil cultivation or mismanagement of forest soil. In this context SOM has received special attention, because of its key role in many important soil processes, its rapid responses to environmental changes, and its feedback to the atmospheric climate system. The total global stock of C in soil is estimated at approximately 2500 Pg C distributed between soil organic C (1550 Pg C) and soil inorganic C (i.e. carbonates, 950 Pg C) (Dungait et al., 2012). The total terrestrial biota contains about 560 Pg C and is the major source of C inputs to SOM pool (Lal, 2008a). Organic carbon is then the largest single component of SOM which encompasses not only the continuum from fresh to progressively decaying plant, microbial and soil faunal debris and exudates but also contains the other elements vital for life, i.e. nitrogen (N), phosphorus (P) and sulphur (S). Terrestrial ecosystems offer significant potential to capture and hold substantially increased volumes of C within SOM, mainly through the recovery of soil C lost due to land-use change (Smith, 2008) and by enhancing the "missing C sink" proposed to exist in soils (Canadell et al., 2007).

One of the most important indicators of soil degradation is the aggregate stability which can be defined as the resistance of the soil against the external destructive effects of rainfall, runoff and wind. More critically, soil erosion rate is substantially controlled by aggregate stability (De Ploey and Poesen, 1985; Farres, 1987; Hairsine and Hook, 1994), and several authors reported that surface erosion processes are linked to aggregate breakdown process by reducing infiltration and improving seal formation (Le Bissonnais and Singer, 1993; Morin and Van Winkel, 1996; Barthes and Roose, 2002; Ramos et al., 2003). It is generally accepted that the soil aggregate stability is affected by two main groups of factors: (1) soil primary characteristics or internal factors, and (2) external factors. These internal factors can be defined by such soil parameters and one of the most important binding agents for forming stable aggregates is soil organic matter (Tisdall and Oades, 1982). The other internal factors are electrolyte concentration of the soil solution, exchangeable cations ($Ca^{2+}$, $Mg^{2+}$, $Na^{+}$ and $K^{+}$) and their relative amount (i.e, sodium adsorption ratio - SAR), clay mineralogy, contents of $CaCO_3$ and gypsum, Fe and Al oxides. Climate, soil formation processes, biological factors and soil managements are among the external factors of affecting soil aggregate stability.

Since direct measurements of the soil susceptibility to the detachment processes on the field level are often expensive and time consuming (Barthes and Roose, 2002; Mutchler et al., 1998), any detailed investigation of the interactions between aggregate breakdown and soil susceptibility to particle detachment would be a more suitable way to estimate and evaluate the soil degradation

tendency in terms of soil erosion. These interactions have been studied by different researchers from past to present, and it has been reported that breakdown mechanisms are closely related to aggregate stability, which is extensively used as an estimator for a force balance between exterior forces exerted on the soil system and interior soil resistive forces to disintegration of structure forming agents (Le Bissonnais, 1996).

Soil erosion process and its impact on ecosystem services receive increasing attention from scientists and policy makers. To assess the environmental security and socioeconomic implications of soil erosion and to develop management plans to deal with them, quantitative and qualitative data on soil erosion rates at regional and global scales are needed (Boardman and Poesen, 2006) and this according to all the steps of soil aggregate breakdown mechanisms that occur during the phenomenon of water erosion. The quantification and qualitative interpretation of the soil aggregates stability indexes in relationship with losses of soil structure cementing agents or soil plants nutrients are of great importance to determine the physical behaviors of soil and to propose the best ways for the soil sustainable management.

## 2.Objectives of the thesis

The analysis of the context and the corresponding bibliography show the needs to determine the quantitative and qualitative relationships between water aggregates stability indexes and losses of soil chemical elements taking into account climate's factors and vegetation type. The general objective of this thesis is therefore to study variation in soil fertility due to the different aggregate breakdown mechanisms and to analyze their interactions in different soil-plant systems, using physical aggregates behavior and organic matter changes as indicators for soil's quality assessment. Specifically, it will be: (i) to quantify the losses of the soil C and major nutrients during the aggregates breakdown process; (ii) to set up the relationship between losses of soil C, nutrients and water aggregates stability; (iii) to better understand the soil structure evolution in specific soil-plant systems. The challenge is then to gain knowledge on the relationships between the evolution of the soil structural stability and the dynamics of SOM associated to soil aggregates under the effects of some factors such as climate's parameters and vegetation type.

## 3.Memory organization

After the general introduction, the thesis begins with a literature review on the research work already done about the climate change, soils and environmental security, where the global

assessment was done on the human activity and its impacts on soils and environment; the sustainable ways to mitigate the effects of world's climate change; the concept of stability of the soil structure, the factors involved in soil aggregation and the water stresses causing aggregate breakdown. The measurement methods of aggregates stability, the dynamics and quantitative relationships with input of organic matter (OM) and ecosystems functions are also exposed. Then, specific objectives and scientific approach that guided this thesis are presented. The results are presented as independent chapters. Therefore, it will follow:

- A chapter on development of a procedure to assess soils aggregates stability and the relative loss of soil nutrients from soil aggregate fractions isolated on the basis of their physical behavior (study area: Farm Ca del Buco);
- A chapter on aggregates stability and loss of soil nutrients in a bio-sequence (study area: Mengone);
- A chapter on bio-climate factors, soil aggregates stability and loss of soil nutrients (study area: Corno alle Scale).

## 4. State of art

### 4.1. Climate changes and soils

Human activities have changed and continue to change the Earth's surface and atmospheric composition. Some of these changes have a direct or indirect impact on the energy balance of the Earth and are thus drivers of climate change (IPCC, 2013).

Climate is an important factor in soil development and is a major driver of the processes of soil formation. At the same time, changes in the bio-physico-chemical properties of soil, due to rising temperatures, changing precipitation intensity and frequency and more severe droughts are really a threat for world's soils. In this context many researches works have been done to analyze how soils will react under changing climate in combination with evolving agricultural and forest practices and how it will be possible in the best way to preserve and store OM in soils in order to mitigate the effects of climate change. Some results of these works concluded that the significant projected changes in precipitation patterns will affect soil formation and functions. Soil as part of the soil-water-plant system contributes and influences changes in groundwater recharge, water quality through buffering capacity, plant growth and evapotranspiration through water availability to roots, and run-off through retention capacity. Changes in rainfall patterns will lead to an increase in erosion in vulnerable soils which often suffer from low organic matter content. For instance, in

recent years, Stanchi et al. (2013) reported that the Aosta Valley (Northwest Italian Alps) has been severely affected by erosion and shallow soil instability. Before, several studies have already established the vulnerability of soils in Italy mainly in changing environmental conditions. According to the Italian Agency for Environmental Protection and Technical Services (APAT, 2009), two thirds of the soils in Italy are affected by degradation problems. Approximately 30% of these soils are potentially prone to erosion rates above the tolerable threshold (Gobin et al., 1999). Specifically, Borrelli et al. (2014), using the Revised Universal Soil Loss Equation (RUSLE) to assess accelerated soil erosion processes of the Italian Apennine intermountain, found that approximately 20.4% of the Turano drainage basin shows only very low soil erosion rates. So, more than 70% of soil of this region are exposed from moderate to high soil erosion rates.

However evaluation of the impact of climate change remains difficult. Changes to features such as texture and mineralogical composition will only occur over long "geological" time spans, while properties such as pH, organic matter content or microbial activity will show a more rapid response. However, effects of warming may be larger in ecosystems that are currently limited by temperature, such as the arctic tundra, the semi-polar deserts (Swift et al., 1998), and mountain areas. In a changing environment, the soil-plant system is fundamental in the regulation of the decay process or decomposition of OM depending of the temperature and directly affects carbon level in soils.

Humic substances were thought to comprise large, complex macromolecules that were the largest and most stable SOM fraction. But, recent advances in physics, material sciences, genomics and computation have enabled a new generation of research on this topic. This in turn has led to a new view of soil-organic carbon dynamics that organic matter persists not because of the intrinsic properties of the organic matter itself, but because of physicochemical and biological influences from the surrounding environment that reduce the probability (and therefore rate) of decomposition, thereby allowing the organic matter to persist. In other words, the persistence of soil organic carbon is primarily not a molecular property, but an ecosystem property. When substrate is available, the decomposition rate of less decomposable substrates appears to respond more to temperature than the decomposition rate of readily decomposable substrates. However, processes controlling substrate availability and the response of those processes to temperature as well as the intrinsic decomposition rate of the less decomposable compounds are also critically important (Kleber et al., 2011). The vulnerability of SOM to degradation will depend on the nature of the disturbance as well as the stabilization and destabilization mechanisms at play in a given ecosystem. Indeed, Conant et al., 2011 has identified three component processes of which variation in rates could affect OM

response to changing environment: (i) depolymerization of biochemically complex compounds; (ii) production and conformation of microbial enzyme production; (iii) and processes that limit the availability of soil OM (adsorption/desorption and aggregate OM occlusion). Regarding the latter aspect, the physical disconnection with decomposition of organic matter is likely to be one of the reason for persistence also of deep SOM (Schmidt et al., 2011). It clearly appears therefore that soil aggregation exerts an effect on the persistence of OM, but also as described in the previous paragraphs, OM is one of the key factors responsible for soil structure development and physical properties (Blanco-Canqui and Lal 2004). In the context of environment variation, this stresses the mutual relationship between physical land degradation and the fate of OM.

### 4.2. Soil and plant systems: preservation of C in forest and agricultural soils

The organic C storage capacity of world soils has been variously estimated for principal biomes (Table 1). Stockmann and collaborators (2013) reported from literature review that approximately 2344 Gt of organic C is already stored in the top three meters of the world's soil, with 54% or 1500 Gt of organic C stored in the first meter of soil and about 615 Gt stored in the top 20 cm. The organic C pool in the first meter of soil represents two third of the global terrestrial C pool and more than the cumulative pool of biosphere and atmosphere (Jobbágy and Jackson 2000). Forest soils, with their high C content in weight terms, should represent a major part of stock (Andreetta et al., 2011). A change of just 10% in the world's soil organic carbon (SOC) pool would be equivalent to 30 years of anthropogenic emissions and could dramatically affect concentrations of atmospheric $CO_2$ (Kirschbaum, 2000). In this context the "good" management of the soil C pool, leading to enhance soil carbon sequestration became the most suitable way to mitigate the negatives effects du to the climate change. Thus, there are a number of overarching questions that need to be considered in relation to the potential of the soil-plant system to "sequester" organic C, where sequestering soil carbon requires a stipulated duration timeframe (usually 100 years) in order to be considered a "permanent" increase under managed forest or agricultural systems. Soil carbon sequestration implies a preservation of soil C for a defined period against a baseline condition where the increased C is sourced from atmospheric $CO_2$.

**Table 1 - Estimates of SOC sink capacity of world soils, listed for principal biomes (from Stockmann et al., 2013)**

| **Biome** | SOC storage (Gt C) by soil depth | | |
|---|---|---|---|
| | 0–1 m | 0–2 m | 0–3 m |
| Tropical regions | 354–403 | 1078–1145 | |
| Other regions | 616–640 | 1760–1816 | |
| **Global estimate** | **1463–1548** | 2376–2456 | |
| Boreal forest | 112 | 141 | 150 |
| Cropland | 157 | 210 | 248 |
| Deserts | 112 | 164 | 208 |
| Sclerophyllous scrubs | 76 | 104 | 124 |
| Temperate deciduous forest | 122 | 145 | 160 |
| Temperate evergreen forest | 73 | 91 | 102 |
| Temperate grassland | 105 | 143 | 172 |
| Tropical deciduous forest | 119 | 175 | 218 |
| Tropical evergreen forests | 316 | 408 | 474 |
| Tropical grassland/savannah | 198 | 281 | 345 |
| Tundra | 114 | 133 | 144 |
| **Global estimate** | **1502** | 1993 | 2344 |
| Boreal forest | 338 | | |
| Cropland | 165 | | |
| Deserts and semi deserts | 159 | | |
| Wetlands | N.A | | |
| Temperate forest | 153 | | |
| Temperate grassland/shrubland | 176 | | |
| Tropical forest | 213 | | |
| Tropical grassland/savannah | 247 | | |
| Tundra | 115 | | |
| **Global estimate** | **1567** | | |
| Boreal forest | 471 | | |
| Cropland | 128 | | |
| Deserts and semi deserts | 191 | | |
| Wetlands | 225 | | |
| Temperate forest | 100 | | |
| Temperate grassland/shrubland | 295 | | |
| Tropical forest | 216 | | |
| Tropical grassland/savannah | 264 | | |
| Tundra | 121 | | |
| **Global estimate** | **2011** | | |

In general, plant production and patterns of biomass allocation strongly influence relative distributions of C with soil depth (Jobbágy and Jackson, 2000). The deeper in the soil profile, the older stored SOC is likely to be. For example, Trumbore (2009) postulated that low-density C and microbial phospholipid acids would increase in age with soil depth. Fontaine et al. (2007) proposed an increase of mean residence times (MRT) of SOC of up to 2,000-10,000 years for depths beyond 20 cm. Increased mean residence times reflect reduced microbial activity and SOC turnover at depth. This conceptual model is supported by patterns of root biomass and relative root density that also decline with soil depth (Jobbágy and Jackson, 2000) and by increasing concentrations of organo-mineral complexes with depth (Fontaine et al., 2007). Schulze et al. (2009) found that the MRT of organic matter was much lower in organic than in organo-mineral horizons of a forest soil, suggesting that the different distributions of SOC described by humus forms may also represent the different SOC distribution among stability pools. The emphasis given nowadays to the capacity that forest soils have to store C originated a new interest in this traditional field of soil science (Bonifacio et al., 2011). This is well demonstrated by the revisions and developments of humus classification systems in several European countries and by the proposal of a worldwide classification of humus forms (Jabiol et al. 2013).

Vegetation type, including dominant tree species, has since long been recognized among the important soil forming factors. Forest floor C stocks are directly affected by litterfall C inputs. According to Augusto et al. (2002), the annual amount of litterfall of a mature temperate forest stand is only slightly influenced by the species of the overstory because the major influences are climate and stand management. Instead, trees species strongly influence C decomposion and consistently higher rates of C mineralization were observed under ash, maple and oak than under Norway spruce (Vesterdal et al., 2012), whereas Olsson et al. (2012) found no difference in mineral soil C mineralization rates under spruce, birch and pine. This synthesis has revealed that tree species indeed influence SOC stocks with quite consistent patterns in forest floor and less consistent patterns in mineral soil. Other researches conclude that there is indeed evidence of consistent tree species effects on SOC stocks. As reviewed by Vesterdal et al. (2013), effects were clearest for forest floor C stocks (23 of 24 studies) with consistent differences for genera common to European and North American temperate forests: Fagus, Quercus, Fraxinus, Acer, Tilia and conifers (Picea, Pinus or Tsuga), and for the boreal forest genera Picea, Pinus, Larix, Populus and Betula. Support for generalization of tree species effects on mineral soil C stocks was more limited, but significant effects were found in 13 of 22 studies that measured mineral soil C. Proportional differences in

forest floor and mineral soil C stocks among tree species suggested that C stocks can be increased by 200–500% in forest floors and by 40–50% in top mineral soil by tree species change.

For targeted use of tree species to sequester soil C, Vesterdal et al. (2013) suggested that the processes related to C input and output, particularly belowground, that control SOC stock differences must be identified. Moreover, they stated that forms and stability of C must be also study in order to assess if certain tree species store C in more stable form.

Regardless of the farming system, e.g. traditional, commercial or modern and innovative, maintenance of SOM is essential to sustaining productivity and minimizing the risks of soil and environmental degradation (Lal, 2006a). There are numerous benefits of increasing the SOM concentration and pool on enhancing ecosystem services and improving the environment. In agricultural soil, the key factor is the improvement in soil quality and C preservation is the application of good management practices. Agricultural soils are of particular importance because about 50% of the total land area of Europe and worldwide are used for agriculture, and there are several management options that promote carbon sequestration in agricultural soils (Smith et al., 2001). Thus, improved management of agricultural soils was included in Article 3.4 of the Kyoto Protocol as a means to reduce atmospheric $CO_2$ levels. Promising agricultural practices are incorporation of manure and crop residues, conservation tillage, set-aside, organic farming, bioenergy and perennial crops, improved management of farmed peat soils, improved pasture and livestock management and conversion of cropland to grassland (Paustian et al., 1997; Smith, 2012). The potential of these practices to increase carbon stocks of agricultural soils has been investigated in numerous publications (Lal, 2008b). As reviewed by Wiesmeir et al. (2013) A rough estimation of the mean amount of crop residues between 1953 and 1997 revealed C stocks increases of 0.5–1.5 t $ha^{-1}$ (+200%) for grain (wheat, barley, rye), 0.5–1.1 t $ha^{-1}$ (+120%) for annual forage crops (corn, sunflower, lupine), 3–4 t $ha^{-1}$ (+33%) for stubble crops (field mustard, rapeseed) and 5–6 t $ha^{-1}$ (+20%) for undersown crops. Root-derived input of organic matter increased from 9 to 15 t $ha^{-1}$ (+67%) for grain, from 10 to 19 t $ha^{-1}$ (+90%) for annual forage crops, from 4 to 7 t $ha^{-1}$ (+75%) for stubble crops and from 11 to 19 t $ha^{-1}$ (+73%) for undersown crops. Large increases of above- and belowground input of organic matter (+114% and +172%) were also observed for perennial forage crops.

### 4.3 Soil physical degradation and key factors controlling this phenomenon

#### 4.3.1 Soil erosion

Soil erosion is a naturally occurring process that affects all landforms. It becomes a problem when human activity or environmental conditions causes it to occur much faster than under natural conditions. Soil compaction, low organic matter, loss of soil structure, poor internal drainage, salinisation and soil acidity problems are other serious soil degradation conditions that can accelerate the soil erosion process. Erosion, whether it is by water, wind or human activity (e.g., tillage), involves three distinct actions: (i) soil detachment, (ii) soil particles movement and (iii) soil particles deposition. Surface horizon materials, which are high in organic matter, fertility and soil life, are relocated elsewhere "on-site" where it builds up over time or is carried "off-site" where it fills in drainage channels. Soil erosion reduces cropland and forest productivity and contributes to the pollution of adjacent watercourses, wetlands and lakes. Soil erosion can be a slow process that continues relatively unnoticed or can occur at an alarming rate, causing serious loss of soil.

Soil erodibility may be defined as the inherent susceptibility of soil to detachment and transport by rain and runoff . Relations exist between aggregate breakdown, which reduces the size of soil fragments, crusting and erosion (De Ploey and Poesen, 1985; Le Bissonnais and Singer, 1993; Hairsine and Hook, 1994). For example Barthes et al. (1999) tried to combine the two aspects and observed that soil susceptibility to erosion is closely related to the topsoil aggregate stability, which is quite easier to assess. Tejada and Gonzalez (2006) in a study on amended soils suggested adopting both erodibility and structural stability as soil vulnerability measures.

Shainberg (1992) observed that slaking, i.e. the aggregate breakdown due to the compression of air entrapped in the pores during rapid rewetting of soils, occurred rapidly under simulated rainfall and was followed by clay dispersion and surface compaction that formed the surface seal. Miller and Baharuddin (1986) found good relations between soil dispersibility and infiltration and erosion. Farres (1987) also stressed that the magnitude of erosion is not just controlled by the breakdown, but also by the size of the detached fragments. In addition aggregate breakdown is responsible for the production of microaggregates and particles, which are easily transported by runoff and splash. This second effect of breakdown is particularly important in interrill erosion, where the runoff detachment capacity is limited. In fact, the size of the detached fragments determines the transportability of fragments.

### 4.3.2 Soil structure

Soil structure refers to the size, shape and arrangement of solids and voids, continuity of pores and voids, their capacity to retain and transmit fluids and organic and inorganic substances, and ability to support vigorous root growth and development. Soil structure is a key factor in the functioning of soil, its ability to support plant and animal life, and moderate environmental quality with particular emphasis on soil C preservation and water quality are well documented. The decline in soil structure is increasingly seen as a form of soil degradation (Chan et al., 2003) and is often related to land use and soil/crop management practices. Soil structure cannot be measured directly, thus it is commonly inferred by measuring the properties of the aggregates. Soil structure is thus often evaluated through aggregate stability that is promoted by organic and inorganic binding agents such as soil organic matter (Tisdall and Oades 1982) clay, carbonates, iron oxides (Bronick and Lal 2005).

### 4.3.3 Soil aggregation and aggregate-SOM models

Aggregates are secondary particles formed through the combination of mineral particles with organic and inorganic substances. The complex dynamics of aggregation is the result of the interaction of many factors including the environment, soil management factors, plant influences and soil properties such as mineral composition, texture, SOC concentration, pedogenic processes, microbial activities, exchangeable ions, nutrient reserves, and moisture availability (Kay, 1990). Aggregates occur in a variety of manners and sizes. These are often grouped by size: macroaggregates (>250 μm) and microaggregates (<250 μm) with these groups being further divided by size (Tisdall and Oades, 1982). Different size groups differ in properties such as binding agents and carbon and nitrogen distribution. There are several mechanisms of aggregation. Aggregates are formed in stages, with different bonding mechanisms dominating at each stage (Tisdall and Oades, 1982). Hierarchical theory of aggregation proposes that microaggregates join together to form macroaggregates and the bonds within microaggregates are stronger than the bonds between macroaggregates (Edwards and Bremner, 1967). Microaggregates are formed from organic molecules (OM) attached to clay (Cl) and polyvalent cations (P) to form compound particles (Cl-P-OM), which are joined with other particles (Cl-P-OM) to form macroaggregates [(Cl-P-OM)x]y (Edwards and Bremner, 1967; Tisdall, 1996). Alternatively, macroaggregates can form around particulate organic matter (POM). As POM is decomposed and microbial exudates are released, the macroaggregate becomes more stable, the C:N ratio decreases, and microaggregates form inside.

The internally formed microaggregates contain more recalcitrant SOC pool (Plante and McGill, 2002). As the more labile SOC pool is utilized and microbial activity decreases, the supply of exudates decreases and the macroaggregate loses stability, eventually disrupts and releases more stable microaggregates (Jastrow et al., 1998; Six et al., 1999).

Roots and hyphae enmesh and release organic compounds that act as glue to hold particles together. Particles can be rearranged during enmeshment, while wet–dry cycles help to stabilize the aggregates. Bacterial microaggregates form as bacterial colonies and their exudates form a polysaccharide capsule around which clay particles are aligned and pulled in by drying and shrinkage. The clay shell forms a protective coating for the bacterial colony inhibiting decomposition of the SOC inside. Concentric theory of aggregation suggests that external layers are concentrically built upon the external surface of the aggregate, with younger C in outer layers of aggregates than in aggregate interiors. The precipitation of hydroxides, phosphates and carbonates enhances aggregation. Cations such as $Si^{4+}$, $Fe^{3+}$, $Al^{3+}$ and $Ca^{2+}$ stimulate the precipitation of compounds that act as bonding agents for primary particles. Cations also form bridges between clay and SOM particles resulting in aggregation. Dissolved organic compounds (DOC) can complex with $Fe^{3+}$ and $Al^{3+}$ at low pH forming mobile, organo-metallic compounds that can be precipitated elsewhere in the soil. The complexation decreases microbial access to SOC and mineralization. It is possible that aggregates form through a combination of these processes (Figure 1).

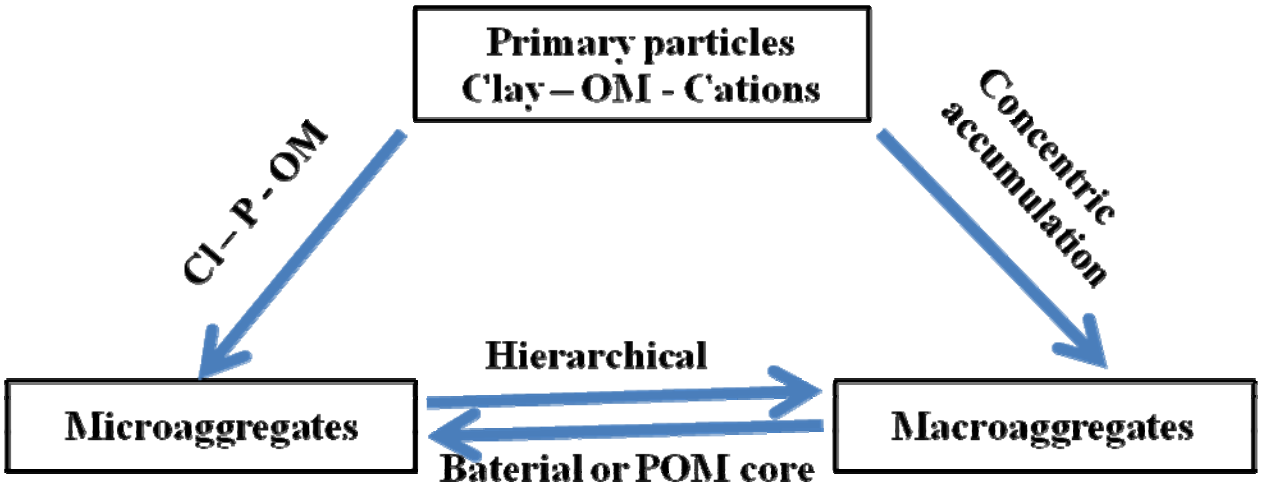



**Figure 1. Some possible scenarios of aggregation according to Bronick et al., (2005). Organic matter (OM), particulate organic matter (POM), clay (Cl), particle (P)**

Macroaggregates may initially form through accumulation of microaggregates or around POM or bacterial cores, decomposing or breaking down later into microaggregates.

Microaggregates may initially form by the progressive bonding of clay, SOM and cations, or as turnover products from macroaggregates.

During the last century several aggregate-SOM models have been proposed as reviewed by Six et al. (2004). Indeed, all major factors playing a role in aggregate formation and stabilization were already identified in the early 1900s. The first aggregate–SOM conceptual model, based on the current theory at that time, was proposed by Emerson (1959). Emerson (1959) described how a soil crumb consisted of domains of oriented clays and quartz particles. According to the Emerson model, SOM increased the stability of a soil crumb by linking together domains of oriented clays and quartz particles. Emerson (1977) further developed his model and argued that the amount of protected SOM is proportional to the surface area of the domains. In other words, only a monolayer of SOM can be associated with the domain surface. This monolayer of SOM is stabilized between clay domains by being cross-linked through Al, Fe and hydrogen bonds. However, the major theoretical stepping stone was proposed by Edwards and Bremner (1967). They presented the microaggregate theory in which the formation of microaggregates was envisioned as a solid-phase reaction between organic matter, polyvalent metals and electrically neutral clays, rejecting Emerson's model of aggregates containing sand grains as primary building blocks. According to Edwards and Bremner (1967), the only highly stable aggregates are fine sand and silt-sized microaggregates (<250 μm) consisting of clay polyvalent metal–organic matter complexes. As describe above, microaggregates are formed by bonding of C-P -OM clay sized units and are represented as $[(C–P–OM)_x]_y$. For the first time they also postulated that the organic matter complexed into the microaggregates would be inaccessible to microorganisms and physically protected.

The aggregate hierarchy concept proposed by Tisdall and Oades (1982) is probably the most significant theoretical advancement in the understanding of aggregate–SOM interactions. In the aggregate hierarchy concept it is postulated that the different binding agents (i.e. transient versus temporary versus persistent binding agents) act at different hierarchical stages of aggregation. Free primary particles and silt-sized aggregates (<20 μm) are bound together into microaggregates (20–250 μm) by persistent binding agents (i.e. humified organic matter and polyvalent metal cation complexes), oxides and highly disordered aluminosilicates. These stable microaggregates, in turn, are bound together into macroaggregates (>250 μm) by temporary (i.e. fungal hyphae and roots) and transient (i.e., microbial- and plant-derived polysaccharides) binding agents. Because of this hierarchical order of aggregates and their binding agents, microaggregate stability is higher and less dependent on agricultural management than macroaggregate stability. Oades (1984) slightly

modified the concept of the hierarchical build up of aggregates. According to the hierarchical aggregate model of Tisdall and Oades (1982), aggregates are sequentially formed, i.e. microaggregates are first formed and then serve as the building blocks for the formation of macroaggregates. Oades (1984) postulated that the roots and hyphae holding together the macroaggregate form the nucleus for microaggregate formation in the center of the macroaggregate.

As a direct consequence of the concept of microaggregates being bound together by SOM into macroaggregates, Elliott (1986) hypothesized that macroaggregates contain more labile SOM than microaggregates and that this SOM is lost upon cultivation. His hypothesis was corroborated and it identified for the first time the direct link between agricultural disturbance, decreased aggregation and loss of labile SOM. Based on these concepts, two sets of observations can be used to identify the existence of an aggregate hierarchy in a soil: (1) an increase in C concentration with increasing aggregate-sized class; and (2) a higher content of new and more labile (e.g. having a higher C:N ratio) C in macroaggregates than in microaggregates.

Based on the concept of microaggregate formation within macroaggregates from Oades (1984), Elliott and Coleman (1988) described, as a mirror image of the aggregate hierarchy, four hierarchical pore categories: (1) macropores; (2) pore space between macroaggregates; (3) pores between microaggregates but within macroaggregates; and (4) pores within microaggregates. This hierarchical pore structure facilitates the understanding of how pore networks determine the links between organisms in a soil food web. The macropores house microarthropods; nematodes move through the pores between macroaggregates; protozoa, small nematodes and fungi inhabit the pore space between microaggregates; bacteria are protected within the pores of the microaggregates. Dexter (1988) formulated the “porosity exclusion principle”. The porosity exclusion principle states that the aggregates of a lower hierarchical order exclude the pore spaces between the building blocks of the aggregates of a higher hierarchical order. Because pores form cavities and failure planes, the excluded porosity in aggregates of a lower order causes their greater density and internal strength compared to aggregates in a higher order.

Oades and Waters (1991) argued that if a soil expresses an aggregate hierarchy, macroaggregates will gradually break down into microaggregates before they dissociate into primary particles, as an increasing dispersive energy is applied to the soil. They observed that in an Alfisol and a Mollisol larger aggregates were progressively broken down into smaller aggregate using increasing intensity of aggregate disruption (i.e., from slow to fast wetting, further shaking and sonication) allowing to illustrate the concept of aggregate hierarchy driven by organic cements, conversely to what occurred for Oxisol, where oxides act as main cementing agent. Oades and

Waters (1991) concluded that aggregate hierarchy exists in soils where aggregate formation and stabilization are directed by organic matter but is not found in oxide-rich soils, where oxides than organic materials are the dominant binding agents and diminish the expression of an aggregate hierarchy.

Golchin et al. (1994) proposed that when fresh plant material (as surface residues or roots) enters the soil, it induces the formation of aggregates because it stimulates the production of microbial-derived binding agents by being a C source for microbial activity. During decomposition, plant material fragments or particulate organic matter (POM) gradually become encrusted with clay particles and microbial products to form the core of stable microaggregates. Microbial mucilages and metabolites further impregnate the mineral crust surrounding the still decomposing organic cores to form very stable microaggregates. Eventually, the organic cores are depleted of available SOM resulting in a cessation of microbial activity and production of mucilages. The halting of production of binding agents and the use of them as substrate leads to a loss of stability of the microaggregates. Upon breakdown of the microaggregates, the mineral crusts impregnated with microbial byproducts are released to form stable silt-sized organo-mineral complexes. Angers et al. (1997) traced C in macro- and microaggregates during decomposition of $^{13}C$-labeled wheat straw under field conditions. They observed that during the initial decomposition period, $^{13}C$ was accumulated in the macroaggregates but the $^{13}C$ enrichment of the macroaggregates decreased thereafter. Concomitant with the decrease in $^{13}C$ in the macroaggregates a stabilization of $^{13}C$ was observed in the microaggregates. This redistribution of C from macroaggregates to microaggregates with time clearly indicates that microaggregates are formed within macroaggregates and then released upon breakdown of the macroaggregates.

### 4.3.4 Soil aggregation agents

- *Soil organic and inorganic carbon (SOC/SIC)*

The source of C, whether it is SOC or SIC, influences its effectiveness in aggregation through associations with cations and soil particles. The SIC exists in soil as primary and secondary minerals. Primary or lithogenic carbonates originate from parent rock material. Primary carbonates are the source material for the formation of secondary carbonates when they are dissolved and translocated by water with organic acids and/or $CO_2$ from soil and atmosphere. Secondary or pedogenic carbonates form when dissolved $CO_2$ precipitates carbonate and bicarbonate with available $Ca^{2+}$ and $Mg^{2+}$ forming carbonate coatings on primary soil particles. The effect of

carbonates on structure is moderated by SOC. Increases in SOC results in increased dissolution of carbonates in soil. However, at low SOC concentration, macroaggregate stability is enhanced by carbonates (Boix-Fayos et al., 2001). On the other hand, high carbonate concentration enhances SOC protection, probably because of decreased SOC mineralization and increased $Ca^{2+}$ (Clough and Skjemstad, 2000).

In the previous paragraph the importance of organic matter as soil aggregation agents has been reported. Here a brief description of the influence of its composition on soil aggregation is done. Indeed, the chemical properties of SOC determine their charge and complexation capacities and influence decomposition rates which have direct effects on aggregation (Schulten and Leinweber, 2000).

The role of polysaccharides in improving soil structure is variable, relating to their source and nature as well as aggregate properties and environmental factors. Polysaccharides are readily mineralizable and act as transient binding agents initiating aggregation, but may not have long-term stability (Kay, 1998; Tisdall and Oades, 1982). Polysaccharides can be strongly adsorbed onto mineral surfaces, acting as ‘‘bridges’’ to bind soil particles (Kay, 1998; Martens, 2000) and prolonging their persistence in soils. Polysaccharides can also form a gel-like substance that acts as a glue to bind particles into aggregates (Haynes and Beare, 1997).

Phenolic molecules can complex with cations to form cationic bridges. Phenols and polyphenols are often regarded as a precursors to stable SOM, such as humic substances (von Lützow et al., 2006). They positively affect soil aggregation, and higher aggregation in soils is often attributed to high phenolic contents in plant residues (Martens, 2000).

Lignin is a high molecular, three-dimensional macromolecule and, after the polysaccharides, it is the most abundant biopolymer in nature (Kögel-Knabner, 2002). Lignin is comparably resistant against microbial decomposion, and only a limited group of fungi (white-rot fungi) is able to completely decompose it to $CO_2$. During biodegradation, lignin undergoes a gradual oxidative transformation process that introduces carboxyl groups in the molecule, so that the transformed molecule is extractable by NaOH and is thus found in the humic fraction (Kögel-Knabner, 2002). The low rate of lignin decomposition is considered a key factor in C turnover rates, enhancing aggregation (Magill and Aber, 1998). Mucilage and other compounds from lignin decomposing fungus contribute to soil aggregation (Caesar-TonThat, 2002).

Lipids are organic substance that are insoluble in water but extractable with non-polar solvents. In soils, lipids are a heterogeneous group of substances that derive both from plants as well as from microorganisms (Kögel-Knabner, 2002). In some soils lipids can improve aggregate stability

against slaking and dispersion, probably related to their hydrophobic nature (Dinel et al., 1997). The effectiveness of lipids in improving aggregate stability may be related to clay mineralogy, as lipids play a more important role in bonding in illite and mixed clay soils than in kaolinite- or smectite-dominated soils (Dinel et al., 1997).

The humic substances, operationally defined on the basis of their solubility, are a heterogeneous soil C fraction. Humic substances are stabilized in soil by humification process and are considered to be refractory, thus belonging to the passive SOM pool (von Lützow et al., 2006). The recalcitrance of humic substances is also a result of their association with the soil matrix, which offers physical protection. Tarchitzky et al. (2000) hypothesized that the distribution of humic compounds, including fulvic acids (FA), humic acids (HA) and humin, in aggregates may reflect the initial coating of particles with humic substances, and then additional humic molecules serving to bond particles together. The HAs can be adsorbed onto clay particles by polyvalent cations, making them especially effective in overcoming clay dispersion, while FAs can interact with metals forming stable organo-Fe or Al associations.

- *Clay and clay mineralogy*

Clay minerals influence properties that affect aggregation: surface area, CEC, charge density, dispersivity and expandability, and these in turn affect SOC decomposition rates (Dimoyiannis et al., 1998; Schulten and Leinweber, 2000). The interaction of clay, SOC and aggregates is affected by soil pH, CEC, ions ($Na^{+}$, $Ca^{2+}$, $Mg^{2+}$), all of which are related to the amount and type of clay present in the soil (Amezketa, 1999). Clay mineralogical composition is modified with soil development (Powers and Schlesinger, 2002). Low-activity clays such as kaolinite and halloysites are often present in Alfisols, Ultisols and Oxisols while high-activity clays with smectites are present in Vertisols. Clays occur as crystalline, layered structures or as non-crystalline minerals with amorphous structure. Non-crystalline clay minerals, such as allophane and imogolite, have high surface areas, and highly variable and pH-dependant charge properties that generally increase aggregation (Powers and Schlesinger, 2002). Non-expanding, crystalline clays, such as kaolinite, have low CEC and surface area, which tend to decrease aggregate stability. Kaolinite has a good flocculation capacity due to electrostatic charges between platelets and SOC. In comparison, aggregation is generally high in high activity clays such as smectites and other 2:1 clays, which are associated with high CEC, large surface areas and high SOC (Amezketa, 1999; Six et al., 2000). The expandability of smectites can disrupt aggregates during wet–dry cycles. Illitic and smectitic

clays tend to decrease aggregate stability during wet-dry cycles although there are variable effects possibly due to the amount of clay and number of shrink-swell cycles.

Dispersivity of soil aggregates is affected by the nature of SOC and the clay type. Cations, particularly $Ca^{2+}$ and $Na^{+}$, electrolytes and pH can influence clay dispersion. Less reactive clays, like kaolinite, are less dispersive. The SOC and some roots can increase dispersion of clay particles while others have the opposite effect (Tisdall, 1996). Negatively charged SOC can be disruptive to aggregate structure, possibly by repelling negative charges in the soil minerals (D'Acqui et al., 1999). Soils dominated by variable charge clay minerals such as 1:1 clay and oxides have higher aggregation at lower SOC levels whereas soils with mixed mineralogy clays have higher aggregation at higher SOC (Denef et al., 2002).

- *Cations*

According to the hierchical model of soil aggregation, cations improve soil structure through cationic bridging with clay particles and SOC. Moreover, as previously mentioned, especially in arid and semi-arid conditions, $Ca^{2+}$ and $Mg^{2+}$ carbonates can precipitate to form secondary carbonate coatings and bind primary soil particles together. Generally, $Ca^{2+}$ is more effective than $Mg^{2+}$ in improving soil structure (Zhang and Norton, 2002). Among bivalent cations, $Ca^{2+}$ can inhibit clay dispersion and the associated disruption of aggregates by replacing $Na^{+}$ and $Mg^{2+}$ in clay and aggregates, thereby adding to aggregate stability. In comparison to $Ca^{2+}$, $Mg^{2+}$ may have a deleterious effect on soil aggregate stability by increasing clay dispersion (Zhang and Norton, 2002). The extent of the negative effect of $Mg^{2+}$ compared to $Ca^{2+}$ may depend on the type of clay and electrolyte concentration in the soil (Zhang and Norton, 2002). Further, $Mg^{2+}$ may result in high swelling by expanding clays, resulting in disruption of aggregates.

Iron and aluminum polyvalent $Fe^{3+}$ and $Al^{3+}$ cations improve soil structure through cationic bridging and formation of organo-metallic compounds and gels (Amezketa, 1999). The solubility and mobility of these cations is pH-dependent, with higher solubility at lower pH. Aggregates containing $Al^{3+}$ and $Fe^{3+}$ and high-CEC clays tend to increase SOC incorporation. The interaction of $Al^{3+}$ and $Fe^{3+}$ with kaolinite can synergistically promote aggregation with limited impact on SOC (Six et al., 2000) while oxides and hydroxides of $Al^{3+}$ interact synergistically with SOC and dispersible clay to improve aggregate stability (Molina et al., 2001). Both $Al^{3+}$ and $Fe^{3+}$ control aggregation in acidic soils with low clay and SOC contents, such as Oxisols. Amorphous $Fe^{3+}$ and SOM form fine stable particles in soils with high SOC content (Barral et al., 1998).

### 4.3.5 Main mechanisms of aggregates breakdown

Aggregate breakdown by water may result from a variety of physico-chemical and physical mechanisms and may involve different scales of soil structure from clay particle interactions to the macroscopic behaviour of aggregates (Tisdall and Oades, 1982). As reviewed by Le Bissonnais (1996), four main mechanisms can be identified from the various reviews already available: (1) slaking; (2) breakdown by differential swelling; (3) breakdown by raindrop impact; (4) physico-chemical dispersion due to osmotic stress.

Slaking is caused by the compression of air entrapped inside aggregates during wetting (Yoder, 1936). It occurs when dry aggregates are immersed in water or rapidly wetted. The effect of trapped air depends on the volume of air inside the aggregates, on the rate of wetting, and on the shear strength of wet aggregates. Slaking occurs even without any shaking of soil in water, although shaking increases the effect of slaking because of additional mechanical breakdown or dispersion. Several authors (e.g., Le Bissonnais, 1996) found that slaking decreases as the initial moisture content increases until saturation is reached.

Differential swelling of clay occurs during wetting. Because of wetting-drying cycles microcracking of aggregates can occur due to the shrinkage and swelling of phyllosilicates (Le Bissonnais, 1996).

Raindrop impact on the soil surface causes mechanical breakdown of aggregates. The fragments resulting from raindrop detachment are generally small, being either elementary particles or small microaggregates (<100 μm) and raindrop impact also displaces these previously detached fragments. This mechanism is called the splash effect. Because very stable macroaggregates can be moved by splash, a discrepancy between aggregate stability and splash measurement under rainfall may be expected.

Physico-chemical dispersion results from the reduction of the attractive forces between colloidal particles while wetting. As previously reported, stability or dispersion depends on cation size and valence. Polyvalent cations cause flocculation, whereas the monovalent cations cause dispersion. The defining characteristic of dispersion is the production of elementary particles rather than microaggregates. Therefore, in clay rich soils, dispersion is one of the most effective processes of aggregate breakdown, and it greatly increases the effect of the others (Bresson and Boiffin, 1990).

The quantification of the soil aggregate stability is of great importance to determine the physical behaviors of soil. Many methods have been devised in order to measure the soil aggregate stability, and there is no satisfactory sole methodology that applies to all soils in any circumstance.

According to Amezketa (1999), the reasons of this are in i) the existence of different mechanisms of aggregate breakdown, ii) the different scale at which stability can be measured, and iii) the different types of methodologies with diverse test protocols. In Table 2 a brief summary of some of the most common methods for the evaluation of the wet aggregate stability is reported.

The ability of a method to give a reliable description of soil physical behavior to different breakdown mechanisms can help in the understanding of the soil erosion dynamics: Shainberg et al. (1992) observed that slow dispersion occurred following rapid slaking during rainfall simulation test; Miller and Baharuddin (1986) found a good relationships between soil dispersibility, water infiltration and soil erosion; Barthés and Roose (2002) indicated slaking as the parameter better correlated to soil erosion measured by rainfall simulator. However, it is not possible that one specific method of analysis could estimate the erosion risk in all soils. However, even if there is not a general agreement, the stability of aggregates is one of the main properties reducing losses of soil by water erosion and thus a useful parameter in the assessment of soil erodibility.

**Table 2 - Some of the most common methods for the evaluation of the wet stability of soil aggregates**

| *Method* | *Aggregate size* | *Expression of result* | *Authors* |
|---|---|---|---|
| Wet-sieving | 3-5 mm | MWD | Yoder (1936) |
| | <2 mm | % of >200 μm (comparison among sample presatureted with ethanol, water and benzene) | Hénin et al. (1958) |
| | bulk soil | change in MWD | De Leenheer and De Boodt (1959) |
| | 1-2 mm | IS (ratio between stable aggregate after 5 and 60' of wet-sieving) | Malquori and Cecconi (1962) |
| | 1-2 mm | % of >250 μm | Kemper and Rosenau (1986) |
| | 2-3.4 mm | MWD | Churchman and Tate (1987) |
| | 3-5 mm | MWD (comparison among fast-wetting, slow-wetting and wet-stirring) | Le Bissonnais (1996) |
| | 1-2 mm | WAS at different time of wet-sieving | Zanini et al. (1998) |
| Raindrop impact | 4-5 mm | time of aggregate rupture | Low (1967) |
| | 2-9 mm | MWD | Young (1984) |
| | 5-8 mm | time of aggregate rupture | Farres (1987) |
| | bulk soil | % of <125 μm | Loch (1994) |
| Ultrasonic dispersion | 4-5 mm | dispersion rate | Edwards and Bremner (1967) |
| | 4-5 mm | pore volume | Grieve (1980) |
| Water immersion | 3-5 mm | Qualitative | Emerson (1967) |
| Dry-sieving | <4 mm | MWD | Kemper and Chepil (1965) |

MWD: mean weight diameter; WAS: wet aggregate stability; IS: stability index

# Chapter II: Development of methodological approach to assess soils aggregates stability and the relative loss of soil nutrients: the case study of mountain agricultural soils (Farm Ca del Buco)

## 1. Introduction

The soil is the main resource for agriculture and it preservation against deterioration or erosion, and the maintenance of the fertilizing elements for crop production are fundamental.

The soil is a reservoir of essential nutrients characterised by their different status, i.e., by their availability together with their concentration at a certain availability level. The availability of a nutrient in soil is the ease with which the nutrient is taken up by plants and it concentration is determined by a competition among the plant root system, the soil solution, and soil solid phases (Sposito, 1989). An available element can exist as an ionic solute in the soil solution and/or adsorbed on soil solid phase. The competition for the solvated-ion in the soil solution involves adsorption and complexation with both inorganic and organic ligands. However, large quantities of nutrient elements persist in forms originally present in soil parent material, particularly in poorly developed soils where processes are not sufficiently expressed to strongly influence soil properties (Bockheim and Gennadiyev 2000), and soil characteristics are largely inherited from the parent material.

In mountain areas soils are often poorly developed and water erosion has a significant impact on the soil fertility since it involves removal and loss of the topsoil layer, where the available nutrient pools are mainly concentrated, and quickly exhausts the nutrient reserves. Despite the importance of the fertility decline due to water erosion, the literature contains few studies on this subject compared with those that evaluate the physical losses of soil and erosion rates (Romero-Dìaz et al., 2012).

The soils at middle elevation are among the most extensive soils for agricultural use in mountain areas, because climatic condition is not a limiting factor, but a large part of these agricultural soils were affected by water erosion as testified by a low or very low organic matter and nutrients content (Costantini et al., 2004). As such, to connect water effect and nutrient cycles, a greater understanding of the water erosion mechanisms and their consequence on the soil nutrients loss is needed.

Aggregate stability is acknowledged for it importance in the protection against soil loss by water erosion (Bronick and Lal, 2005), and it is defined as the resistance of the soil particles to the structural rearrangement when exposed to stresses (Cass, 1999). The abundance and stability of the

aggregates are even critical for several soil functions, such as plant growth, resistance to erosion (Le Bissonnais and Arrouays, 1997; Barthès and Roose, 2002), soil organic matter turnover (Six et al., 2000), presence, activity and diversity of organisms, both mesofauna (Quénéhervé and Chotte, 1996) and microflora (Six et al., 2006). The most common methods for its evaluation are based on Yoder's (1936) wet sieving procedure (e.g., Kemper and Rosenau, 1986; Williams et al., 1966). They quantify the loss of stability caused by wetting (slaking, swelling and dispersion) and the shaking actions (water abrasion) as a whole. The difficulties with these procedures are both to standardise the methods and that soils have different structural stabilities and react differently to the different water stress phenomena (i.e., wetting and shaking actions). The use of pretreatments has been thus suggested to elucidate the mechanisms and processes involved in disaggregation (e.g. Hénin et al., 1958; Le Bissonnais, 1990). Le Bissonnais (1996) proposed a method of wet sieving after prewetting with ethanol to limit the breakdown of aggregates due to fast wetting and discriminate between the losses due to wetting and shaking action. Sample pretreatments can discriminate the single aggregate breakdown mechanism allowing to detect the relevance of different soil properties with respect to the aggregate breakdown mechanisms and to differently rank soils on the basis of their physically-based aggregate behaviour (Amezketa et al., 1996). 1997). For instance, Oades and Water (1991) observed that in an Alfisol and a Mollisol larger aggregates were progressively broken down into smaller aggregate using increasing intensity of aggregate disruption (i.e., from slow to fast wetting, and further shaking) allowing to illustrate the concept of aggregate hierarchy driven by organic cements, conversely to what occurred for Oxisol, where clay particles act as main cementing agent

According to Baker and Laflen (1983), nutrients may be lost by water action in three ways: percolation through the soil profile, being dissolved in the runoff water, and adsorption to the sediment carried by the runoff water. During aggregate wet sieving tests, similar processes can lead to the loss of dissolved nutrients and those adsorbed or present in the solid phase. Thus, in order to determine both the aggregate breakdown and the nutrients losses due to the two main mechanisms of disaggregation (i.e., fast wetting and abrasion), in this study we analyse the nutrients contained in the water and in the soil solid phase generate by the aggregate rupture due to wet sieving procedure applying sample pretreatments. The objectives of this study are:

- to evaluate the nutrients status and the loss of fertility suffered by some mountain agricultural soils in the northern Italian Appennine, and

- as the availability of nutrients in the topsoil is expected to directly influences the concentration of these nutrients in the water and soil particulate, we also try to establish a relation between the original characteristics of the soils and the losses by wet sieving.

The sampling site is agrarian and since 2004 had known history of organic cultivation, neither nutrients nor organic matter inputs were supplied. The soils developed on calcareous shales. As such, the site is able to provide a natural baseline for nutrients availability and aggregate properties in similar soils and parent materials, as well as yield insight into relationships between loss of soil fertility and water aggregate stability in calcareous mountain regions.

## 2. Materials and Methods

### *2.1 Study area and soil sampling*

Three soil profiles (A, B and C), representative of the some of the most common soil types of agricultural land in the northern Italian Appennine, were described and sampled in the Monte Pastore area (500 m a.s.l.) near to Bologne (44°22' N, 11°08'E). The soil developed on shales and calcilutites (E-R Ambiente, Servzio geologico sismico e dei suoli, 2006). The sampling sites were chosen on a moderate slope (15%). The area has a temperate warm subcontinental climate; the mean annual rainfall is 820 mm and the mean temperatures ranges from 10 to 15°C. The soil moisture and temperature regime was ustic and mesic respectively. According to the World Reference Bases of soil resources (IUSS Working Group WRB, 2006), the soils were classified as Leptic Regosol (site A) and Colluvic Regosols (sites B and C). In Table 1, the main characteristics of soil profiles were reported.

In the study area, since 2004 crops rotation was practised with certified organic wheat, peas, barley and clover. Each year, soil structure was disturbed by tillage with mouldboard plough, working to 20 cm depth, for the control of weeds.

In autumn 2010 after tillage of fields, two pits in each site were dug and soil samples were taken from the 0-20 cm topsoil of each pit.

### *2.2 Main physico-chemical characteristics of soils (<2mm) and aggregates (1-2mm)*

Topsoil samples were air-dried and sieved to pass a 2-mm screen obtained the so called <2 mm fine earth fraction. Then, the 1-2 mm aggregates were separated from the fine earth fraction by

dry sieving to 1 mm and weighted. Both the <2 mm fine earth and 1-2 mm aggregates fractions were analysed for the main physico-chemical characteristics.

The pH was determined potentiometrically in a 1:2.5 soil to deionised water suspension. The texture was determined by the pipette method after dispersion of the sample with a sodium hexametaphosphate solution (Gee and Bauder 1986). The carbonate content was measured by volumetric analysis of the carbon dioxide liberated by a 6 M HCl solution (Loeppert and Suarez, 1996). The total Fe and Al concentrations (Alt and Fet) were measured by ICP-OES after $HNO_3$: HCl (1:3) microwave-digestion of sample. The dithionite-citrate extractable Al and Fe (Ald and Fed; Mehra and Jackson, 1960 ) were determined by ICP-OES.

### *2.3 Nutrient status of soils (<2mm) and aggregates (1-2mm)*

The nutrient status were determined in the <2 mm fine earth and 1-2 mm aggregates fractions of the topsoil.

Total organic C ($C_{org}$) and total nitrogen (N) were determined by dry combustion (Thermo Scientific Lab EA-1110) on carbonate-C free samples. The concentration of soluble elements ($Ca_{sol}$, $Mg_{sol}$, $K_{sol}$, $Na_{sol}$ and $P_{sol}$) was measured by ICP-OES in a 1:2.5 soil to deionised water suspension, after centrifugation and filtration of the water extracts (van Reeuwijk, 2002). The concentration of exchangeable cations ($Ca_{exch}$, $Mg_{exch}$, $K_{exch}$, $Na_{exch}$) and the cation exchangeable capacity (CEC) were determined by ICP-OES after exchange with 0.05 N cobaltihexamine chloride solution (Orsini and Rèmy, 1976, modified by Ciesielski and Sterckeman, 1997). The P available ($P_{Olsen}$) was extracted with 0.5 M sodium bicarbonate solution at pH 8.5 (Olsen and Sommers, 1982) and the P concentration was measured by ICP-OES. The total element concentrations ($Ca_t$, $Mg_t$, $K_t$, $Na_t$, and $P_t$) were measured by ICP-OES after $HNO_3$:HCl (1:3) microwave-digestion of sample.

### *2.4 Aggregate stability and losses of nutrients*

For the standard test of aggregate water stability, 10 g of the 1–2 mm aggregates were placed in 0.2-mm sieves and gently immersed in water for 10 min (fast wetting). Thereafter, the aggregates were wet sieved at 60 cycles $min^{-1}$ in beakers containing deionized water (water abrasion). The sieving time was set at 10 min. After the completion of the sieving period, the material remaining in the sieves was oven dried at 40°C and weighted. The water aggregate stability ($WAS_T$) related to fast wetting and water abrasion was determined as follows:

$$WAS_T = \frac{weight\ retained - weight\ of\ coarse\ sand}{total\ sample\ weight - weight\ of\ coarse\ sand} \cdot 100$$

The coarse sand was determined on the 1–2 mm aggregate fraction after $H_2O_2$ oxidation. The percentage of aggregate total loss ($Loss_T$) was calculated as 100-$WAS_T$. To test the wet cohesion independently from the breakdown due to water saturation (i.e., fast wetting) and thus to determine the wet aggregate stability against the abrasion, 10 g of the 1–2 mm aggregates were placed in sieves and gently immersed in 95% solution of ethanol for 10 min (Le Bissonnais, 1996) and then wet sieved for 10 min. The material remaining in the sieves was oven dried, weighted and the stability indexes related to the abrasion ($WAS_{ABR}$ and $Loss_{ABR}$) were calculated. From the difference between $WAS_T$ and $WAS_{ABR}$, or $Loss_T$ and $Loss_{ABR}$, we calculated the breakdown caused by fast wetting ($WAS_{FW}$ and $Loss_{FW}$, respectively; Falsone and Bonifacio, 2006).

**Table 1 - Description of soil profiles according to Schoeneberg et al. (2002)**

| Site | Horizon | Depth | Munsell colour | structure [a] | | | Texture [b] | roots [c] | | WRB soil classification |
|---|---|---|---|---|---|---|---|---|---|---|
| | | cm | Dry | G | S | T | | Q | S | |
| **A** | Ap | 0-24/25 | 2.5Y 3/2 | 2 | co | sbk | C | 2 | vf,f | **Leptic Regosol** |
| | CB | 24/25-32/33 | Gley 5/5GY, 10YR 7/8 | 1 | co | sbk | cl | 1 | f | |
| | CR | 33-70+ | Gley 5/5GY | 1 | co | sbk | cl | 0 | f | |
| **B** | Ap1 | 0-28/35 | 7.5YR 3/1 | 3 | co | sbk | C | 2 | vf,f | **Colluvic Regosol** |
| | Ap2 | 28/35-60/65 | 10YR 3/2 | 3 | vco | sbk | C | 1 | f | |
| | Ab | 60/65-80 | 10YR 3/2 | 2 | m | sbk | cl | 0 | f | |
| | C | 80-95+ | 2.5 YR 4/3 | 2 | m | sbk | cl | 0 | f | |
| **C** | Ap1 | 0-25/28 | 10YR 4/4 | 2 | co | sbk | C | 2 | vf,f | **Thapto-Endogleic Colluvic Regosol** |
| | Ap2 | 25/28-54/60 | 2.5Y 5/4 | 2 | co | sbk | cl | 1 | f | |
| | Ab | 54/60-67/70 | 10YR 4/3 | 2 | co | sbk | cl | 0 | f | |
| | Bgb | 67/70+ | 10YR 5/4 | 2 | co | sbk | cl | 0 | f | |

[a] structure: **(G)** Grade, 1=weak, 2=moderate; 3=strong; **(S)** Size, m=medium, co=coarse, vco=very coarse; **(T)** Type, sbk=subangular blocky; [b] texture: c=clay, cl= clay loam; [c] roots: **(Q)** Quantity, 0=very few, 1=few, 2=common; **(S)** Size, vf=very fine, f=fine

From the standard test of aggregate stability, we determined the loss of C, N, P, K, Ca, Mg, and Na due to fast wetting and water abrasion (Figure 1). The excess water in beakers, containing the material passed through the sieve after 10 minutes of water immersion (i.e., elements losses due to fast wetting) was centrifuged at 10.000 rpm and filtrated <0.45 μm to separate the liquid and solid phases. In the liquid phase (in Figure 1 (2)), the organic C and N lost as soluble forms (Csol and Nsol) were measured by a TOC-TN analyzer (Shimadzu, TOC-V/CPN), the elements in aqueous solution ($P_{sol}$, $K_{sol}$, $Ca_{sol}$, $Mg_{sol}$, $Na_{sol}$) were determined by ICP-OES. In the solid phase (in Figure 1 (3)), the organic C and N lost as particulate ($C_{par}$ and $N_{par}$) were determined by dry combustion (Thermo Scientific Lab EA-1110) on carbonate-C free samples and element concentrations ($P_{par}$, $K_{par}$, $Ca_{par}$, $Mg_{par}$, $Na_{par}$) were measured by ICP-OES after $HNO_3$:HCl (1:3) microwave-digestion of sample. The nutrients lost were labelled as fast wetting susceptible (FW). An aliquot of the excess water in beakers, containing the material passed through the sieve after 10 minutes of wet sieving (i.e., elements losses due to abrasion), was centrifuged at 10.000 rpm and filtrated <0.45 μm to isolate the liquid phase. The C, N, P, K, Ca, Mg, and Na lost as soluble forms were measured as described above and labelled as abrasion susceptible (ABR; in Figure 1 (4)). On the remaining material after 10 minutes of water immersion and 10 minutes of wet sieving, we determined the organic C and N content by dry combustion ($C_{stab}$ and $N_{stab}$), the element concentrations ($P_{stab}$, $K_{stab}$, $Ca_{stab}$, $Mg_{stab}$, $Na_{stab}$) after $HNO_3$:HCl (1:3) microwave-digestion of sample, and they represents the element stable to both fast wetting and abrasion .(in Figure 1 (5)) From the difference between the nutrients concentration in the sample before the stability tests (in Figure 1 (1)), the elements stable, the elements susceptible to fast wetting and the soluble elements susceptible to abrasion, we calculated the elements losses as particulate due to abrasion (in Figure 1 (6)).

**Figure 1 - Scheme of the sequence of fractionation of nutrients loss**

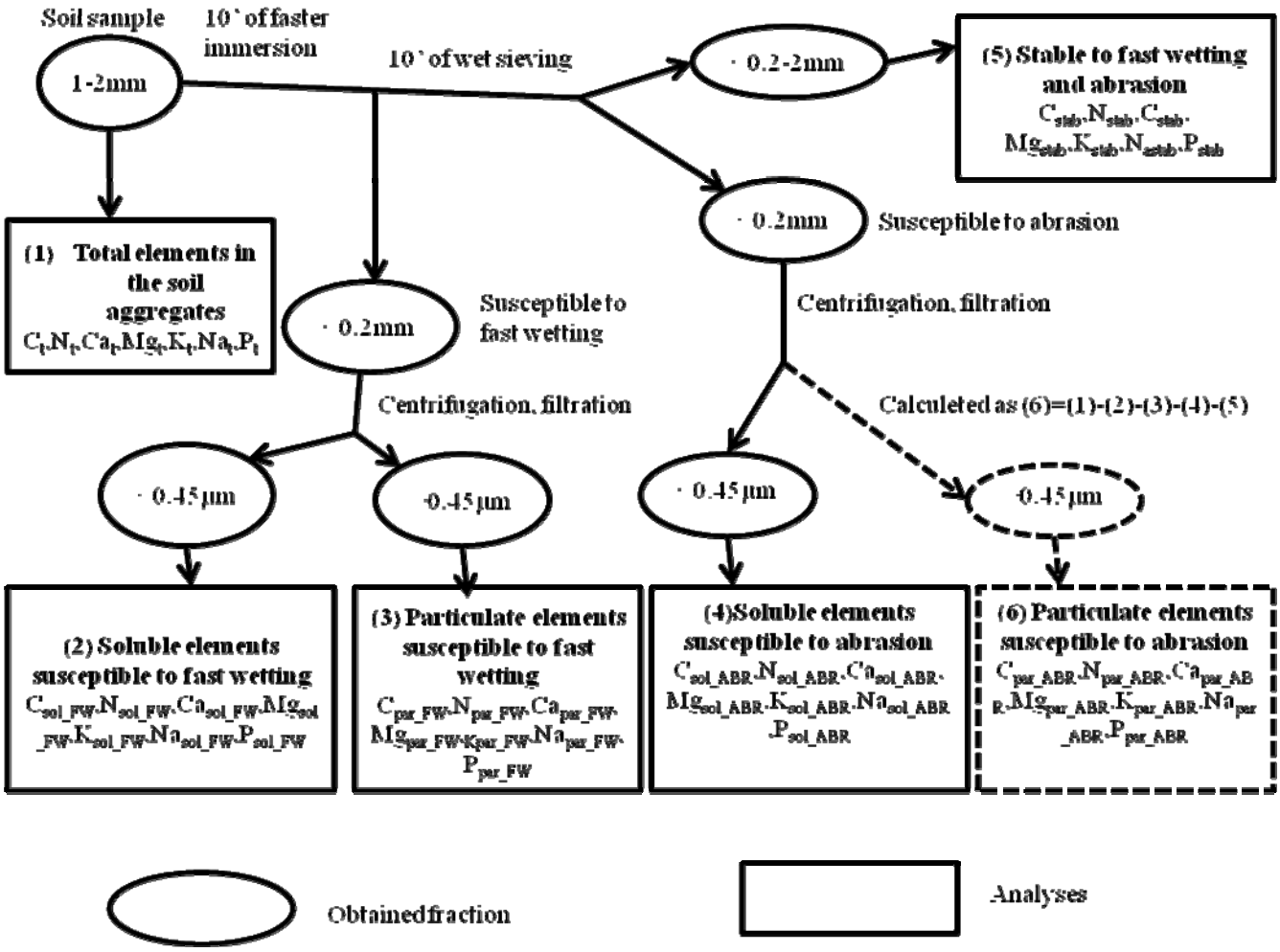


**Table 2. Fine earth (<2 mm) and aggregate (1-2 mm) properties of the topsoil. In parentheses, the standard deviation is given. Coefficient of variation is always lower than 10% with the exception of CaCO3, whose coefficient of variation is up to 42%**

| Fraction | Site | pH | $CaCO_3$ | $C_{org}$ | N | $C_{org}/N$ | $Fe_d$ | $Al_d$ | $Fe_d/Fe_t$ | $Al_d/Al_t$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | g $kg^{-1}$ | | | | g $kg^{-1}$ | | | |
| **<2 mm** | A | 8.3 | 88 | 13.2 | 2.0 | 6.8 | 12.7 | 2.1 | 0.22 | 0.03 |
| | B | 8.3 | 49 | 18.1 | 2.5 | 7.4 | 10.4 | 1.6 | 0.18 | 0.02 |
| | C | 8.4 | 37 | 14.4 | 1.8 | 8.0 | 12.8 | 1.7 | 0.24 | 0.03 |

| Fraction | Site | $Ca_{sol}$ | $Mg_{sol}$ | $K_{sol}$ | $Na_{sol}$ | $P_{olsen}$ | CEC | $Ca_{exch}$ | $Mg_{exch}$ | $K_{exch}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $mgL^{-1}$ | | | mg $kg^{-1}$ | $cmol_c kg^{-1}$ | | | |
| **<2 mm** | A | 24.7 | 2.7 | 3.6 | 3.3 | 2.24 | 29.3 | 25.9 | 2.3 | 0.3 |
| | B | 26.6 | 2.5 | 2.2 | 5.0 | 2.51 | 35.2 | 32.4 | 2.3 | 0.3 |
| | C | 33.3 | 3.4 | 1.9 | 7.4 | 2.26 | 34.7 | 30.8 | 2.4 | 0.3 |

| Fraction | Site | % of aggregates | $CaCO_3$ | $C_{org}$ | N | $Fe_d$ | sand | coarse silt | fine silt | Clay |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | g $kg^{-1}$ | | | | | | | |
| **1-2 mm** | A | 39.1 | 110 | 12.3 | 1.8 | 11.4 | 227 | 65 | 305 | 398 |
| | B | 42.4 | 60 | 17.6 | 2.3 | 11.7 | 208 | 64 | 317 | 421 |
| | C | 40.0 | 50 | 15.2 | 2.0 | 13.7 | 214 | 71 | 314 | 407 |

| Fraction | Site | $Ca_{sol}$ | $Mg_{sol}$ | $K_{sol}$ | $Na_{sol}$ | $P_{olsen}$ | CEC | $Ca_{exch}$ | $Mg_{exch}$ | $K_{exch}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $mgL^{-1}$ | | | mg $kg^{-1}$ | $cmol_c kg^{-1}$ | | | |
| **1-2 mm** | A | 35.3 | 4.1 | 3.1 | 4.5 | 2.18 | 30.0 | 25.5 | 2.0 | 0.3 |
| | B | 31.0 | 2.7 | 2.3 | 5.7 | 2.46 | 36.1 | 31.5 | 2.2 | 0.3 |
| | C | 29.4 | 3.0 | 1.8 | 5.8 | 1.90 | 35.1 | 31.3 | 2.5 | 0.3 |

## 3. Results

### *3.1 Fine earth and aggregate properties*

The fine earth fraction (<2 mm) from the topsoil was moderately alkaline, with a calcium carbonate contents varying from 37 and 88 g $kg^{-1}$ (Table 2). The content of organic C was homogenous among the sites (from 13.2 to 18.1 g $kg^{-1}$) as well as the $C_{org}/N$ ratio (from 6.8 to 8.0). The pedogenetic Al oxides content was very low, as was expected given the low degree of soil development, and represented only the 3% of the total Al content ($Al_d/Al_T$). The amount of $Fe_d$ represented the 22% of the total Fe content without any differences among sites.

The amounts of 1-2 mm aggregates was about 40% of the fine earth (Table 2). Their texture was clay. The aggregate properties were very similar to those of corresponding fine earth fraction and they were enriched neither in organic C nor in $CaCO_3$ or Fed with respect to the fine earth.

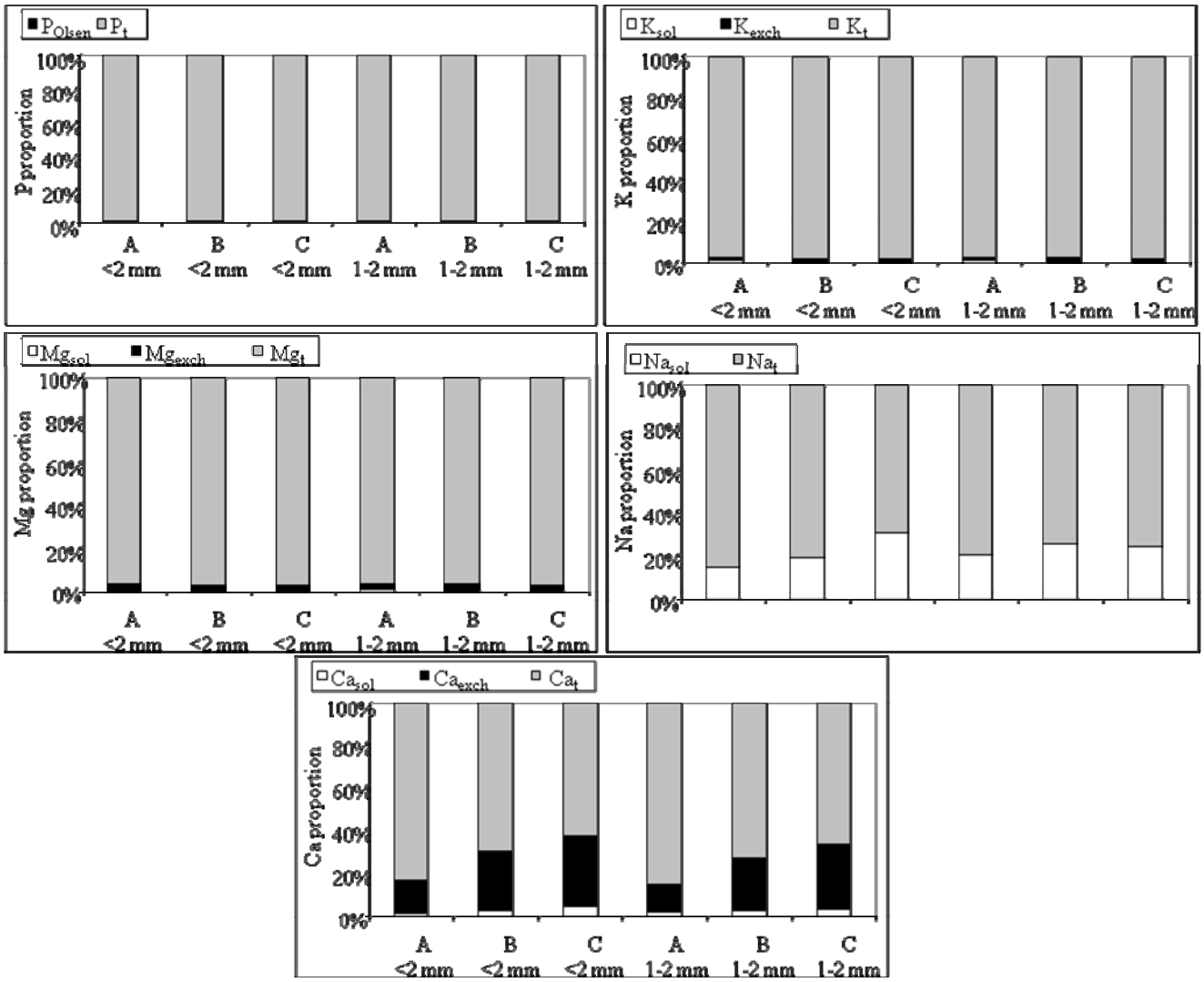
As regards the nutrients content, the N value of both fine earth and aggregates was on the average 2 g $kg^{-1}$ and was parallel to that of organic C, suggesting that N in inorganic form (available to plants) was lower than organic form. The organic C content also affected the CEC ($r^2$=0.80, $p<0.01$).

The pools of the P, K, Ca, Mg and Na showed that the distribution between easily available form (i.e, soluble) and exchangeable form varied according to the element species and again in a similar way between the fine earth and aggregates (Table 2 and Figure 2). Given the presence of calcareous materials, the available P was very low and represented less than 1% of total P (Figure 2). Also the K and Mg were mainly present as total form, and only the 2-3% were available (of which 0.7% was as soluble form). The available Na was as Na soluble, and it accounted for 15-30% of the total Na; in contrast the content of available Ca was high and it was mainly as exchangeable Ca (13-33% of total Ca).

### *3.2. Aggregate stability and soil losses*

The wet aggregate stability index ($WAS_T$; Table 3) was 57% in the A site and about 80% in the B and C sites. The A site was more fragile than B and C sites both to the fast wetting and water abrasion, as showed by the amount of soil lost by fast wetting and abrasion ($Loss_{FW}$ and $Loss_{ABR}$, respectively; Table 3). However, the relative percentage of abrasion, thus the incidence of water abrasion on the total soil losses, showed that for all the sites the most relevant mechanism of aggregate breakdown was the abrasion (always >60%).

**Figure 2 - Mean proportion of nutrient pools fractionated on the basis of their chemical availability in the fine earth (<2 mm) and aggregates (1-2 mm) fractions. Coefficient of variation is always lower than10%**




The total loss of each element (Σ total loss; Table 4) was calculated from the sum of the concentration of the nutrient lost as soluble and particulate due to the fast wetting and the water abrasion (Table 4). On the average, the total loss of C from the aggregates was 3.1 g $kg^{-1}$ of the initial mass of aggregates, the loss of N was 0.6 g $kg^{-1}$, P was 140 mg $kg^{-1}$, and K 2.3 g $kg^{-1}$ (Table 4). The losses of the other elements were: Ca 9.5 g $kg^{-1}$, Mg 2.4 g $kg^{-1}$, and Na 0.6 g $kg^{-1}$. With respect to the initial element concentration in the aggregates, the losses greatly varied among nutrients: they accounted for 20% of C, 27% of N, 22% of P, 24% of K, 33% of Ca, 22% of Mg and 96% of Na. This occurred in all sites, independently from their aggregate stability, suggesting a nutrient species effect on elements losses.

**Table 3 - Aggregate stability indexes. In parentheses, the standard deviation is given. Coefficient of variation is always lower than 10%**

| Site | $WAS_T$ % | $Loss_T$ % | $Loss_{FW}$ % | $Loss_{ABR}$ % | $RP_{ABR}$ %[a)] |
|---|---|---|---|---|---|
| A | 57.3 | 42.7 | 16.6 | 26.1 | 61.4 |
| B | 80.9 | 19.1 | 2.4 | 16.7 | 87.4 |
| C | 79.5 | 20.5 | 4.4 | 16.1 | 78.6 |

[a)] $RP_{ABR}$: relative percentage of water abrasion, calculated as the ratio between $Loss_{ABR}$ and $Loss_T$

The loss of nutrients and total loss of aggregates ($Loss_T$) were significant correlated in the case of P, K and Mg (0.93, 0.92 and 0.93, respectively; Table 5). The same was found for $Loss_{ABR}$.

Although for all sites the most relevant mechanism of aggregate breakdown was the water abrasion, the total fertility loss due to the water abrasion was not significant related to the $Loss_{ABR}$ (Table 5). This was due to the different behaviour among nutrients. In fact, the amount of element lost due to the abrasion (soluble and particulate) varied among nutrient species (Table 4), and it accounted for >80% of the total loss for K, Ca and Mg, but <55% for C, N and Na. The abrasion affected in an intermediate way the loss of P (64% on average). Consequently, our findings pointed to relatively high losses of C, N, Na, and partly of P, due to fast wetting even if the incidence of fast wetting on the total soil aggregates losses was low.

In the case of C and N, the loss of fertility due to fast wetting was related to the amount of aggregates lost during water immersion ($Loss_{FW}$; r=0.99, Table 5). The same happened for P (r=0.81, Table 5).

As regards elements form, all the nutrients were mainly lost as particulate (always >80% of the total loss), with the exception of N and Na for which the loss as soluble form was high (on the average 63 and 48% of the total loss, respectively; Table 4). However, if we took into account the different nutrients pools on the basis of their initial chemical availability (i.e., soluble, exchangeable and total elements), the amount of loss of any nutrient was not related to the initial element content in the aggregates.

**Table 4. Fractionation of nutrients pools on the basis of their physical stability. Data are expressed as g or mg of nutrient $kg^{-1}$ of initial mass of aggregates**

| Nutrient | nutrient pools [a] | A site | B site | C site | Average | | |
|---|---|---|---|---|---|---|---|
| | | loss of nutrient pool | loss of nutrient pool | loss of nutrient pool | Σ total loss[b] | Σ loss due to fast-wetting (FW) [c] | Σ loss due to water abrasion (ABR)[d] |
| C g $kg^{-1}$ | $C_{sol\_FW}$ | 0.26 | 0.21 | 0.53 | 3.09 | 1.37 | 1.72 |
| | $C_{sol\_ABR}$ | 0.28 | 0.28 | 0.22 | | | |
| | $C_{par\_FW}$ | 2.04 | 0.43 | 0.65 | | | |
| | $C_{par\_ABR}$ | 1.53 | 0.51 | 2.31 | | | |
| N g $kg^{-1}$ | $N_{sol\_FW}$ | 0.03 | 0.02 | 0.53 | 0.55 | 0.35 | 0.2 |
| | $N_{sol\_ABR}$ | 0.10 | 0.03 | 0.02 | | | |
| | $N_{par\_FW}$ | 0.32 | 0.06 | 0.09 | | | |
| | $N_{par\_ABR}$ | 0.10 | 0.10 | 0.24 | | | |
| P mg $kg^{-1}$ | $P_{sol\_FW}$ | bdl | bdl | bdl | 140 | 50 | 90 |
| | $P_{sol\_ABR}$ | bdl | bdl | bdl | | | |
| | $P_{par\_FW}$ | 105 | 16 | 29 | | | |
| | $P_{par\_ABR}$ | 144 | 47 | 79 | | | |
| K g $kg^{-1}$ | $K_{sol\_FW}$ | 0.04 | 0.02 | 0.06 | 2.3 | 0.49 | 1.81 |
| | $K_{sol\_ABR}$ | 0.04 | 0.14 | 0.07 | | | |
| | $K_{par\_FW}$ | 0.71 | 0.23 | 0.42 | | | |
| | $K_{par\_ABR}$ | 3.01 | 0.69 | 1.46 | | | |
| Ca g $kg^{-1}$ | $Ca_{sol\_FW}$ | 0.19 | 0.23 | 0.18 | 9.47 | 1.36 | 8.12 |
| | $Ca_{sol\_ABR}$ | 0.65 | 0.65 | 0.62 | | | |
| | $Ca_{par\_FW}$ | 1.25 | 0.53 | 1.69 | | | |
| | $Ca_{par\_ABR}$ | 5.79 | 9.20 | 7.46 | | | |
| Mg g $kg^{-1}$ | $Mg_{sol\_FW}$ | 0.02 | 0.01 | 0.02 | 2.43 | 0.32 | 2.11 |
| | $Mg_{sol\_ABR}$ | 0.04 | 0.05 | 0.04 | | | |
| | $Mg_{par\_FW}$ | 0.20 | 0.26 | 0.45 | | | |
| | $Mg_{par\_ABR}$ | 4.77 | 0.67 | 0.76 | | | |
| Na g $kg^{-1}$ | $Na_{sol\_FW}$ | 0.03 | 0.07 | 0.01 | 0.56 | 0.32 | 0.23 |
| | $Na_{sol\_ABR}$ | 0.19 | 0.27 | 0.24 | | | |
| | $Na_{par\_FW}$ | 0.23 | 0.24 | 0.39 | | | |
| | $Na_{par\_ABR}$ | bdl | bdl | bdl | | | |

[a] Nutrient pools codes reported according to those in Figure 1

[b] Σ total loss: sum of the concentration of the loss of nutrient pools

[c] Σ loss due to fast-wetting: mean of soluble and particulate elements lost due due to fat-wetting

[d] Σ loss due to water abrasion: mean of soluble and particulate elements lost due to water abrasion

bdl: below detection limit

**Table 5. Pearson's coefficient correlation (r) among loss of aggregates due to different aggregate breakdown mechanism (i.e., total breakdown – T, water abrasion – ABR, and fast wetting – FW) and the loss of nutrient (C, N, P, K, Ca, Mg, Na and total) due to the same mechanisms**

| Total breakdown | | | water abrasion | | | fast wetting | | |
|---|---|---|---|---|---|---|---|---|
| Aggregate loss | element loss [a)] | r | Aggregate loss | element loss [b)] | r | Aggregate loss | element loss [c)] | r |
| | C | 0.447 | | C | 0.152 | | C | 0.976** |
| | N | 0.786 | | N | -0.069 | | N | 0.996** |
| | P | 0.932** | | P | 0.909* | | P | 0.813* |
| $Loss_T$ | K | 0.915* | $Loss_{ABR}$ | K | 0.857* | $Loss_{FW}$ | K | 0.767 |
| | Ca | -0.247 | | Ca | -0.217 | | Ca | 0.408 |
| | Mg | 0.934** | | Mg | 0.769* | | Mg | -0.196 |
| | Na | -0.379 | | Na | -0.729 | | Na | 0.066 |
| | Σ total | 0.563 | | Σ total | 0.446 | | Σ total | 0.735 |

[a)] element loss= $element_{sol\_FW}$+ $element_{sol\_ABR}$+ $element_{par\_FW}$+ $element_{par\_ABR}$

[b)] element loss= $element_{sol\_ABR}$+ $element_{par\_ABR}$

[c)] element loss= $element_{sol\_FW}$+ $element_{par\_FW}$

Σ total: sum of the element losses; **: $p<0.01$; *: $p<0.05$

## 4. Discussion

### *4.1 Nutrient status*

The studied soils were Regosols, thus very weakly developed soils marked by incipient soil formation whose characteristics were largely inherited from parent material (IUSS Working Group WRB, 2006). The aggregates of the three studied sites did not differ in term of chemical properties from the fine earth fraction, confirming that chemical properties of the 1-2 mm aggregates well represent those of bulk soil.

The amount of total P was in the usual reported range of soil P (from 500 to 800 mg $kg^{-1}$; Stevenson, 1986) and it represented about 99% of the P in the soils. Due to calcareous origin of soils, the soil pH was above the pH of insolubility of Ca phosphates, therefore the P plant-available form was low, and soil P mainly occurred as insoluble form (Sims and Pierzynski, 2005). Parent material composition strongly influenced also soil cation nutrient status: the soil solution and exchangeable sites were Ca-dominated, whereas Mg and K in soil solution and to exchange sites accounted for <3%. Similar results were found by Stutter et al. (2003) that investigated the content of exchangeable base cations in soils derived from different parent material, ranging from acidic granite to basic limestone. They found high content of Ca and low of Mg in calcareous soils,

showing a close relation to the parent material. Among the major nutritional cation in soil, the available K accounted for very small proportions of the total K. The concentration of K in the soil solution, which was in the reported range for agricultural soil of temperate regions (from 2 to 5 mg $l^{-1}$; Haby et al., 1990), was too low to meet the requirement of K by a crop during growing season (Huang, 2005). With the exception of Ca, the other element inherited from parent material were thus few available. The content of organic C in these soils was also low and affected the organic N fractions and the cation exchangeable capacity of these soils. Therefore, in these soils organic matter control the largest reservoir of potentially available N in soils (Stevenson, 1994) and the concentration of available cations (Sposito, 1989). Therefore, low organic C content suggested a low availability of these soil nutrients. Thus, in addition to soil development degree and parent material, also the agricultural use and soil organic matter management further affected the nutrient status in the studied soils. Neither nutrients nor organic matter inputs were supplied and the organic C in these soils derived totally from the remains of plants.

*4.2 Relation among soil original characteristics, water aggregate stability and loss of soil fertility*

On the basis of chemical availability of nutrients, it was expected high losses of P, K, Ca and Mg as particulate, and high loss of Na as soluble form. Of all the nutrients required for plant growth, N is by far the most mobile and subject to greatest loss form the soil system (Stevenson, 1994). It is well known that in agroecosystems a great amount of N is lost by leaching, mainly as $NO_3^-$, but also as dissolved organic N (McLauchlan, 2006). Therefore, even if we did not make any speciation of N, we expected a certain loss of N as soluble form. This initial hypothesis was partly confirmed by the data of loss of fertility. In fact, as regards the quality of losses, N and Na had great losses as soluble forms. On the contrary the other nutrients were mainly lost as particulate. However, from the quantitative point of view, the loss of nutrients was not related to the initial concentration of nutrients in the aggregates. We found instead strong links between the loss of aggregate mass and nutrient losses.

A high proportion of C was lost during fast wetting, and the amount of C lost was related to the quantity of solids released, suggesting that C-rich materials are lost immediately. It is well known that soil organic C acts as a binding agent and as a nucleus in the formation of aggregates, affecting their stability (Bronick and Lal, 2005) and therefore the losses of C-rich materials are often limited. However, in agricultural soils, tillage disrupts soil aggregates, lowering soil organic C positive effects, and enables soil erosion (Plante and McGill, 2002). As a consequence, organic C is

lost through erosion and transferred downslope or to the water (McLauchlau, 2006). In our studied soils, the C was lost mainly as particulate, therefore the risk of C transfer from soil system to the water seemed to be of few relevance. On the contrary, a great amount of N was lost as soluble form. These findings partly confirmed our initial hypothesis on fertility losses formulated on the basis of the chemical data, however, also for N, the loss was linked to the amount of solids released during fast wetting. Therefore, C and N-rich materials were lost immediately due to the wetting alone. The P loss also partly confirmed the initial hypothesis: P was lost as particulate, because of its association with soil particles (Sims and Pierzynski, 2005). We also found a good correlation between the release of P and the aggregates losses, both due to fast wetting and abrasion. It could thus suggested that part of P-rich materials are lost immediately and that additional material broken down during wet sieving further contributes to P loss.

As expected from the chemical data, particulate forms also prevailed in the loss of K, Mg and Ca. Over the life cycle of a field crop, most of the K, Mg and Ca would be supplied by ions absorbed to the exchange complex of soil particles (Hossner, 2008). Therefore, both unavailable and available forms of basic cation were lost during aggregate breakdown. Water abrasion mainly contributed to the loss of these elements, and K and Mg released were related to the loss of aggregates. This suggested that the materials released during wet sieving are K and Mg-rich.

Even if Na is not among the chemical elements essential to plant growth in soil, during the aggregate breakdown process it was noted that its behaviour greatly varied from those of the other basic cations. First of all, about 50% of Na was lost as soluble form. Moreover, Na was quite totally lost. It is often reported the worsening effect of Na on the soil structure properties (Bronick and Lal, 2005): the $Na^+$ is a highly dispersive agent resulting directly in the breakdown of aggregates and indirectly affected aggregation; in soil solution and at exchange sites Na contributes to repulsive charges that disperse soil particles. In our soils, it could be possible that particle surrounded by $Na^+$ were preferentially detached or dispersed and consequently Na was preferentially lost. Large Na loss occurred during fast wetting, probably because dispersion results from the reduction of attractive forces between colloidal particles during wetting (Emerson, 1967; Sumner and Stewart, 1992).

## 5. Conclusions

With the exception of Ca, the studied soils were poor in available nutrients. The type of parent material on which they develop, the weak degree of their evolution and their agricultural use affected the soil fertility status.

The availability of nutrients in the topsoil directly influences the quality of nutrients losses, but did not influence the quantity of nutrients lost. This latter was instead affected by the severity of aggregate breakdown due to water saturation and/or water abrasion. However, the mechanisms of aggregate breakdown affected the amount of loss of fertility in a different way among nutrients. In particular P, K and Mg were the most susceptible to water abrasion. Instead C, N and P also were mainly susceptible to wetting, and important loss C, N and P occurred during water immersion.

These findings suggest a general high risk of soil nutrient depletion, and that even rainfall of low intensity, but sufficient to water saturated the topsoil, may lead to further decrease in the fertility status of these soils. The protection of the surface is crucial in these soils. The preservation of soil cover might help in this way by acting as a mechanical barrier against rapid soil saturation and abrasion energy of soil water. The proposed combined method (i.e, determination of aggregate stability and loss of nutrients) based on physical behaviour of aggregates seems to be useful in the mechanistically understanding of the soil C and nutrients fate related to erosion and it might be suggested for studying the impact of management on soil C and nutrients, aggregates properties and their relationships.

# Chapter III: Aggregates stability and loss of soil nutrients in a Chestnut-Douglas fir biosequence (Mulino Mengone site, Italy)

## 1. Introduction

Plant species affects the content and chemical composition of soil organic matter, and influences fertility properties such as pH, cation exchange capacity and the equilibrium between pools of nutrients (Binkley and Giardina 1998; van Breemen and Finzi 1998). Tree species was also the most important explanatory variable for the C:N ratio in both forest floor and topsoils; for instance Cools et al. (2014) found the lowest C:N ratios both in the forest floor and the top mineral soil in black locust (*Robinia pseudoacacia* L.) and black alder (*Alnus glutinosa* L.) stands, both N fixing tree species, while in the forest floor the highest C:N ratios were found in evergreen species like pine, cork oak (*Quercus suber* L.) and eucalyptus, the pine species and Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) showed the highest C:N ratios in the mineral soil. Among the latter, Douglas fir is the most productive tree species (Podrázský et al., 2013). For this reason Douglas fir was first introduced to Europe from North America more than 150 years ago, was then planted on a large scale and is now the economically most important exotic tree species in European forests (Schmid et al., 2014). When compared with domestic coniferous species, Douglas fir proved to have lower acidifying effects on upper soil layers and contributes to better humus forms, recycling nutrients more effectively and producing litter which could be easily decomposed (Kupka et al., 2013). However, compared to broadleave species, Strobel (2001) found that the content of low molecular weight carboxylic acids in soil and soil solution increased from deciduous forest to coniferous forest, and that in particular Douglas fir caused higher contents of organic acid extractable, such as oxalic acid.

The quali-quantitative properties of primary SOC input derived from plant biopolymer residues also strongly affect its stabilisation (e.g., von Lützow et al., 2006). According to the SOC properties, part of the C input is either decomposed to $CO_2$ or transformed into more stable humic substance through the humification process (Stevenson, 1994). However, other processes act in SOC turnover such as interactions with soil minerals and occlusion within soil aggregates (e.g., von Lützow et al., 2006). Soil aggregates, on the other site, were stabilised by SOC because of the cementing capacity of organic compounds (Bronick and Lal, 2005). By applying a combination of chemical characterisation and aggregate physical fraction, it should therefore be possible to determine the effect of SOM properties on aggregate stability and the capacity of soil aggregate to

limit SOC, or other elements, depletion. A method for aggregate physical fraction is based on the aggregate resistance against water stress, and thus on the aggregate physical resistance against water saturation and abrasion (Le Bissonnais, 1996; see also Chapter 1). The method aims to isolate stable aggregates to slaking (i.e., aggregate explosion due to fast wetting) and wet sieving.

Based on these assumptions, stabilisation of SOC depends on the interaction between aggregate-associated OC properties and water aggregate stability. The chemical composition and origin of SOC seem to be very important in establishing these relationships. Because of great replacememts of indigenous species with Douglas fir plantations in European forests (Schmid et al., 2014), studies on the fate of Douglas fir OC need.

In this study we thus selected a Chestnut-Douglas fir biosequence, where Chestnut plants were partly replaced with Douglas fir for sanitary reason, in order to i) analyse the changes in the properties of aggregate-associated OC due to the different plant species and ii) determine their effect on aggregate stability and loss of OC and of other elements.

## 2. Materials and Methods

### *2.1. The study area*

Mulino Mengone is a hamlet located in the Northern Apennines in the Monghidoro municipality of the province of Bologna and the chosen study area is at 925-935 m asl, northwest oriented (between 308 and 317°N) on a gentle slope of 11-15%. The parent material belongs to the sandstone facies of the Monghidoro formation, with variable grain size from coarse to fine (Gazzi, 1966, Valloni and Zuffa,, 1984; Cipriani et al., 1985). The siliciclastic component is dominant and it is composed by clasts of quartz, feldspars and micas. The annual mean temperature of the area is 10°C, while the mean rainfall ranges between 1110 and 1460 mm. The soil temperature regime is mesic, and the soil moisture regime is udic.

The study area is actually dominated by European chestnut (*Castanea sativa* Mill.) wood, and in 1963-1964 about 3.5 ha were reforested with Douglas fir. At present the chestnut forest and Douglas fir reforestation were adjacent and three site were clearly distinguished on the basis of the dominant tree cover and litter composition forming a well-defined biosequence (Figure 1): a Chestnut dominated wood whose litter was composed by leaves, branches, burrs and other plant residues clearly came from chestnut trees (CS site); a transitional zone on the border of reforestation dominated by Douglas fir trees and with litter composed by both Chestnut and Douglas fir residues

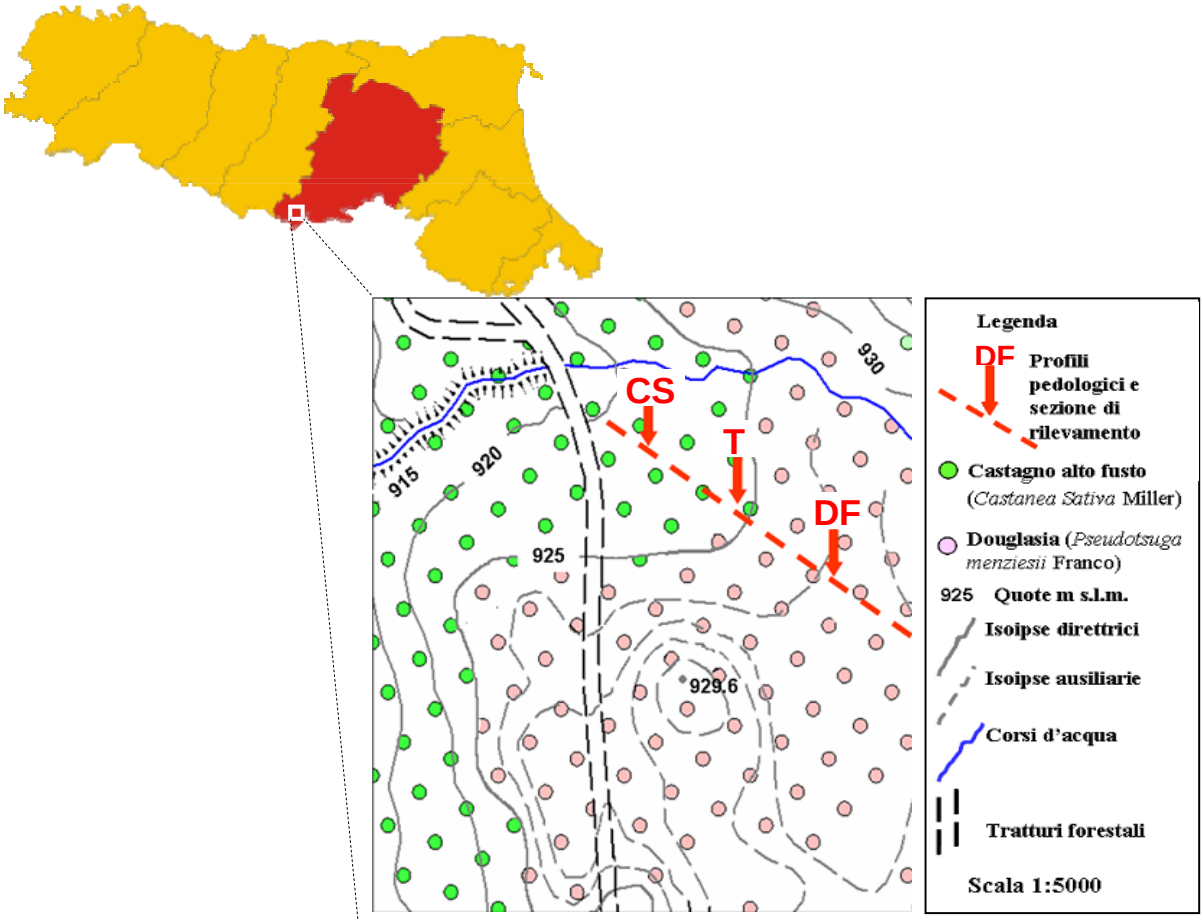
(T site); a Douglas fir reforested zone with a litter layer composed by needles, branches and other residues came from Douglas fir trees (DF site).

In the summertime 2011, a representative soil profile for each site was open. The three soil profiles were dug at 10 m intervals along a 20 m transect. (Figure 2). According to the WRB (IUSS, ISRIC, FAO 2006) the soil of CS site was classified as Haplic Regosol (Humic, Eutric), while the soils of DF and T sites as Haplic Cambisols (Humic, Eutric).

**Figure 1** – The studied biosequence

CS site T site DF site

**Figure 2 -** Localization of the study area and soil sampling scheme



*2.2. Aggregate fractionation*

Bulk soil samples were collected from each genetic mineral horizon of the representative soil profiles. Soil samples were air-dried to constant weight and dry-sieved to pass a 2-mm screen (<2 mm). Then, the 1-2 mm aggregates (AG) were separated from the fine earth fraction by dry sieving to 1 mm and weighted. A weighted aliquot of AG was placed in 0.2-mm sieve and wetted by immersion in water for 10 min (i.e., water saturation), then wet sieved at 60 cycles $min^{-1}$ (i.e., water abrasion). At the end of wet sieving, the aggregates remaining in the sieves were oven dried at 40°C, weighted and their amount was expressed as percentage of AG (MA). This separated fraction represented the macroaggregates stable to water stress (i.e., water saturation and abrasion).

*2.3. Measurement of the 1-2 mm aggregate stability to the different breakdown mechanisms*

The wet stability of the 1-2 mm aggregates to different aggregate breakdown mechanism was determined by Yoder's (1936) wet sieving procedure (Kemper and Rosenau, 1986) using pretreatments as a way to elucidate the mechanisms and processes involved in aggregate breakdown (Hénin et al., 1958; Le Bissonnais, 1990).

The aggregate stability to water saturation and abrasion ($WAS_t$) was determined as follows

$$WAS_t = \frac{MA - coarse\ sand}{100 - corse\ sand} \cdot 100$$

$WAS_t$, MA and coarse sand were expressed as percentage. The coarse sand fraction (>0.2-mm) was determined on 1-2 mm aggregate after $H_2O_2$ oxidation.

To test the wet cohesion independently from the breakdown due to water saturation and thus determine the wet aggregate stability to water abrasion, 10 g of 1-2 mm aggregates were placed in sieves and gently immersed in 95% solution of ethanol for 10 min (Le Bissonnais, 1996) and then wet sieved for 10 min at 60 cycles $min^{-1}$. The material remaining in the sieve was oven dried, weighted and the stability index related to the abrasion ($WAS_{ab}$) was calculated as follows

$$WAS_{ab} = \frac{weight\ of\ retained\ materials - weight\ of\ coarse\ sand}{10\ g\ of\ aggregates - weight\ of\ coarse\ sand} \cdot 100$$

The weight of coarse sand fraction (>0.2-mm) was determined as described above.

*2.4. Main physico-chemical properties of aggregates*

The 1-2 mm aggregates were characterised for their main physico-chemical properties, in particular those properties involve in the soil aggregation and nutrient status. The pH was determined potentiometrically in a 1:2.5 (w:v) soil-deionised water suspension for mineral or organic horizons (Van Reeuwijk, 2002). The texture was obtained by the pipette method after dispersion of the sample with a sodium hexametaphosphate solution (Gee and Bauder 1986). The total organic carbon (OC) and total nitrogen (N) contents were measured by dry combustion (EA-1110 Thermo Scientific Lab). The exchangeable cations ($Ca^{2+}$, $Mg^{2+}$, $Na^{+}$, $K^{+}$) were determined by Inductively Coupled Plasma Optical emission Spectrometry (ICP-OES, Amtek, Arcos Spectro) after exchange with 1M $NH_4^{+}$-acetate at pH 7 (Summer and Miller, 1996).

Both the interaction of $Al^{3+}$ and $Fe^{3+}$ with SOC, as organo-metallic compounds, and oxides-hydroxides of Fe can promote aggregation in acidic soils (e.g., Six et al., 2000). Therefore, the amount of both amorphous Al and Fe forms and well-crystalline pedogenic Al and Fe oxides was determinated. Amorphous iron (Feo) and aluminium (Alo) forms were estimated through extraction with acid-ammonium oxalate (Schwertmann, 1964), and Fe and Al in the extracts were measured by ICP-OES. Pedogenic iron oxides (Fed) were estimated through extraction with Na–dithionite–citrate–bicarbonate (Mehra and Jackson, 1960) and Fe in the extracts was analysed by ICP-OES. The total element concentrations ($Al_t$, $Fe_t$, $Ca_t$, $Mg_t$, $K_t$ and $P_t$) were measured by ICP-OES after $HNO_3$:HCl (1:3) microwave-digestion of sample.

*2.5. Humic carbon extraction from aggregates*

The humic C (HC) was extracted from the 1-2 mm aggregates with 0.5 M NaOH. Because no further separation of humic compound was performed, here the term HC is comprehensive of both humic and fulvic fraction. An aliquot of extracted HC was used to determined the amount of C by wet oxidation at 160 °C with 1/3M $K_2Cr_2O_7$, according to the method Springer and Klee (1954). The rest of HC was dialysed, freeze-dried and stored for further analysis.

*2.6. Micro-attenuated total reflectance (ATR) infrared spectroscopy*

The ATR/FT-IR spectra of different soil horizons and their extractable humic carbon (HC) fraction were recorded using a Nicolet 5700 Thermo-Corporation interferometer equipped with a diamond attenuated total reflectance (ATR) accessory (Spectra-Tech, Shelton, CT), by co-addition of 100 scans at a resolution of 4 $cm^{-1}$ in the range 4000-400 $cm^{-1}$. A background spectrum using the diamond crystal only was recorded prior to collection of each sample spectrum. In order to better understand the main features of aggregates organic composition (both total organic matter and humic fraction), the IR spectra have been examined in the region between 1800-1300 $cm^{-1}$. The bands near 1730 and 1376 $cm^{-1}$ have been assigned to C=O stretching vibration of ester, such as uronic acid and acetyl groups in hemicellulose (Nuopponen et al., 2006). However ester carbonyl groups found in hemicellulose have the stretching vibrations at higher frequency (≈1750 $cm^{-1}$) whereas lignin tends to have more ketone/aldehyde-type carbonyl groups (1730 $cm^{-1}$) (Orton et al., 2004), therefore we can not exclude in this region also the contribute of lignin functional groups. The bands located at 1630 and 1420 $cm^{-1}$ are mainly attributed to asymmetrical and symmetrical stretching in carboxylates and OH vibration of water between phyllosilicate layers. In addition other groups such as C=O stretching in quinones, amide I, and aromatic structures vibrations can partly contribute to the first band (1630 $cm^{-1}$) vibration. The shoulder band at 1530 $cm^{-1}$ is assigned to aromatic C=C stretching. Many FTIR analyses of different types of lignin and wood materials have assigned this band as a major band of lignin (Pandey, 1999). Therefore we have taken the peaks at 1530 $cm^{-1}$ as a measure of the lignin component.

For better characterizing the HC, which typically showed a set of overlapped peaks, a curve fitting analysis was performed. The second-derivative of IR spectra in the region from 3000-2800 $cm^{-1}$ and 1800-1100 $cm^{-1}$ regions, smoothed with a 10-point smoothing by Savitzky–Golay function, were used to define some starting parameters for the curve fitting analysis, such as the number and position of peak components by the Grams/386 spectroscopic software (version 6.00, Galactic Industries Corporation, Salem, NH). The IR spectra were fitted using bands of Gaussian shape. The optimum Gaussian curve-fitting was determined by the lowest value for the minimization function of reduced Chi square ($\chi 2$) and a good agreement between experimental and calculated profiles (coefficients of determination (R2) between 0.999 and 0.988 and the standard error (SE) in range of 0.001-0.0003).

*2.7. Elements concentration of water stable macroaggregates*

The total organic carbon (OC) and total nitrogen (N) contents of MA were measured by dry combustion, while the concentration of Al, Fe, Ca, Mg, K, and P were measure by ICP-OES after $HNO_3$:HCl (1:3) microwave-digestion of sample as described above. They represent the element of macroaggregates stable to water saturation and abrasion.

*2.8. Statistical analysis*

All statistical analyses were carried out using SPSS software package (SPSS Inc., Chicago, IL). The correlations were estimated using Pearson's or Spearman's coefficients depending on the linearity of dependence evaluated by visual inspection of the data. A probability level of 0.05 was always used as threshold for significance.

**3. Results**

*3.1. Soil aggregate distribution*

The amount of 1-2 mm aggregates (AG) varied from 12 to 43% and, as visible in Figure 3, independently from the vegetation cover type the highest amount was in the A2 horizon, thus in the A horizon below surface horizon. The amount of aggregate stable to fast wetting and water abrasion (MA) varied from 41 and 98% and it showed a progressive decrease along soil depth (Figure 3).

*3.2. Aggregate properties related to soil aggregation process*

Aggregates were acidic (Table 1). Independently from vegetation type, aggregates from the surface horizon (A1) were rich in organic matter (SOM >88 g $kg^{-1}$) and in the underlying horizon (A2) the SOM content sharply decreased to then progressively decline down to 4-12 g $kg^{-1}$. Clay content varied from 71 to 165 g $kg^{-1}$, and generally increased with depth. Exchangeable cations, especially Ca, had high values both in surface and deeper horizons (A1 and CB, respectively). Higher amounts of amorphous Al and Fe oxides (Alo and Feo) were found in DF and T soils than in CS. Along soil depth, A2 or AB horizons had the highest Alo contents, while the content of pedogenic Fe oxides (amorphous –Feo or well-crystalline -Fed) was high in Bw horizons (Table 1).

In order to identify the relationships between aggregate formation and cementing agents, correlations between the amount of AG or MA and aggregate properties were checked. Because in forest soil the positive effect of organic matter on formation and stabilization of aggregates is well documented (e.g., Bronick and Lal, 2005), at first we checked the relation between SOM content and AG, MA amounts (Figure 4a and b). In general, an increase in AG and MA occurred accordingly to the increase in SOM, and if A1 horizons (i.e., those with SOM> >88 g $kg^{-1}$) were excluded from the relationships, strong linear correlation were found ($r=0.825$ and 0.840, respectively; $p<0.01$). Similarly, we checked the relationships between AG, MA and the other aggregate properties. As visible in Table 2 , Alo content was the only variable positively related to MA when A1 horizons were excluded from the dataset ($r=0.735$, $p<0.01$), while pH value, clay content and exchangeable-Ca were negatively related to the MA.

**Figure 3** – Distribution of percentage of 1-2 mm aggregates (AG) and macroaggregates stable (MA) to water stress (i.e., water saturation and abrasion) in the studied sites

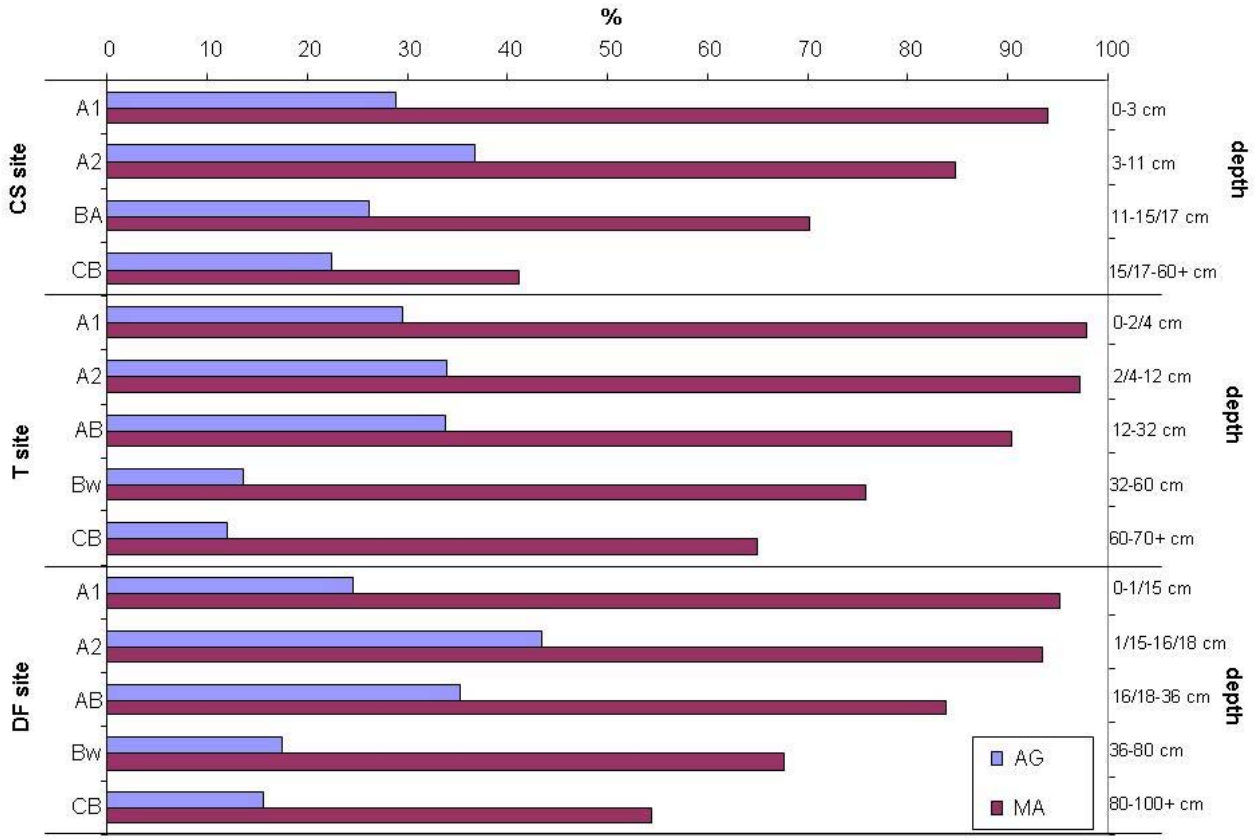


**Table 1 –** Main physico-chemical properties of the 1-2 mm aggregates

| Site | Horizons | pH | SOM | clay | exchangeable cations § | | | Alo | Feo | Fed |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Ca | Mg | K | | | |
| | | | g $kg^{-1}$ | | $cmol_+$ $kg^{-1}$ | | | g $kg^{-1}$ | | |
| CS | A1 | 4.4 | 115.5 | 108 | 2.92 | 1.10 | 0.28 | 1.93 | 2.82 | 5.69 |
| | A2 | 4.8 | 20.9 | 90 | 0.63 | 0.25 | 0.07 | 2.17 | 2.87 | 5.42 |
| | AB | 5.0 | 9.4 | 109 | 0.59 | 0.25 | 0.05 | 1.63 | 2.48 | 5.17 |
| | CB | 5.3 | 4.0 | 152 | 2.09 | 0.48 | 0.09 | 1.13 | 1.75 | 6.13 |
| T | A1 | 4.1 | 88.1 | 18 | 1.63 | 0.51 | 0.17 | 1.93 | 2.57 | 4.74 |
| | A2 | 4.6 | 25.2 | 86 | 0.35 | 0.19 | 0.07 | 2.17 | 2.45 | 5.00 |
| | AB | 4.7 | 24.8 | 99 | 0.25 | 0.18 | 0.06 | 2.92 | 3.02 | 5.74 |
| | Bw | 4.7 | 12.0 | 136 | 0.23 | 0.16 | 0.04 | 2.45 | 3.32 | 7.01 |
| | CB | 4.9 | 7.8 | 116 | 0.25 | 0.17 | 0.04 | 1.36 | 1.58 | 4.09 |
| DF | A1 | 4.3 | 93.8 | 71 | 3.48 | 0.91 | 0.28 | 2.07 | 2.50 | 5.87 |
| | A2 | 4.6 | 38.4 | 104 | 0.54 | 0.37 | 0.11 | 2.85 | 2.93 | 6.97 |
| | AB | 5.0 | 27.7 | 104 | 0.47 | 0.37 | 0.06 | 2.55 | 2.91 | 5.85 |
| | Bw | 5.3 | 17.0 | 146 | 0.84 | 0.38 | 0.06 | 2.56 | 3.22 | 6.25 |
| | CB | 5.3 | 12.1 | 165 | 1.37 | 0.37 | 0.06 | 1.97 | 3.12 | 5.05 |

SOM: soil organic matter determined by multiply the content of organic C by 1.72; § amount of exchangeable-Na was always <0.01 $cmol_+$ $kg^{-1}$

**Figure 4** – Relationships between the amount of SOM and (a) percentage of 1-2 mm aggregates (AG) and (b) macroaggregates stable (MA) to water stress

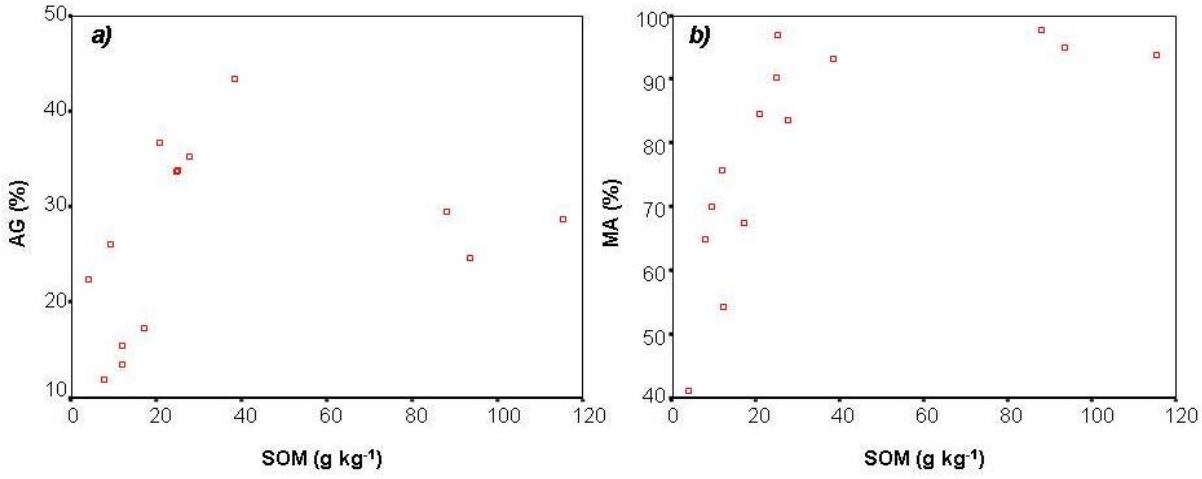


**Table 2** – Spearman ($r_s$) and Pearson's (r) correlation coefficients ~~of~~ between the amount of aggregates (AG), water stable macroaggregates (MA) and soil properties.

| all soil horizons | | | without A1 horizons | | |
|---|---|---|---|---|---|
| | $r_s$ AG | MA | | r AG | MA |
| pH | -0.385 | -0.895** | pH | -0.538 | -0.832** |
| clay | -0.657* | -0.908** | clay | -0.740** | -0.844** |
| exchangeable-Ca | -0.037 | 0.108 | exchangeable-Ca | -0.211 | -0.789** |
| exchangeable-Mg | 0.059 | 0.218 | exchangeable-Mg | 0.081 | -0.516 |
| exchangeable-K | 0.521 | 0.657 | exchangeable-K | 0.690* | 0.197 |
| Alo | 0.451 | 0.292 | Alo | 0.491 | 0.735** |
| Feo | 0.064 | -0.029 | Feo | 0.177 | 0.399 |
| Fed | 0.029 | -0.138 | Fed | 0.232 | 0.143 |

*: $p<0.05$; **: $p<0.01$

### *3.3. Total element concentration in the aggregates*

Total element concentration in the aggregates (AG) was reported in Table 3. The OC content decreased along soil depth varying from 67.2 to 2.3 g $kg^{-1}$. N contents ranged from 4.7 to 0.4 g $kg^{-1}$, the topsoil of DF and T sites had lower N content than CS, and in all soils N followed the trend of SOM (or OC). On the average, the DF site had higher P content than CS and T sites (401, 284 and 274 mg $kg^{-1}$), even if the maximum P concentration (601 mg $kg^{-1}$) was in the aggregates from A1 horizon of CS site. Similarly to the exchangeable form, total Ca content had high values both in surface and deeper horizons (A1 and CB, respectively), while total Mg increased along soil depth. The content of K, Al and Fe was on the average 3.4, 23.2 and 15.7 g $kg^{-1}$ and no clear trend was detected along soil profile.

**Table 3** – Total element concentration of the 1-2 mm aggregates (AG)

| Site | Horizons | OC | N | $P_t$ | $Ca_t$ | $Mg_t$ | $K_t$ | $Al_t$ | $Fe_t$ |
|---|---|---|---|---|---|---|---|---|---|
| | | g $kg^{-1}$ | | mg $kg^{-1}$ | g $kg^{-1}$ | | | | |
| CS | A1 | 67.2 | 4.7 | 601 | 1.21 | 3.35 | 3.08 | 22.0 | 14.7 |
| | A2 | 12.2 | 1.3 | 255 | 0.43 | 3.58 | 3.59 | 24.8 | 16.5 |
| | AB | 5.4 | 0.8 | 157 | 0.38 | 3.44 | 2.78 | 20.1 | 14.7 |
| | CB | 2.3 | 0.4 | 126 | 0.66 | 4.91 | 4.23 | 25.3 | 19.6 |
| T | A1 | 51.2 | 2.9 | 288 | 0.83 | 2.69 | 2.92 | 19.3 | 12.2 |
| | A2 | 14.7 | 1.5 | 229 | 0.24 | 3.28 | 2.92 | 20.5 | 14.2 |
| | AB | 14.4 | 1.3 | 327 | 0.27 | 3.35 | 3.13 | 23.5 | 15.3 |
| | Bw | 7.0 | 0.9 | 339 | 0.29 | 3.27 | 3.22 | 22.1 | 15.2 |
| | CB | 4.5 | 0.5 | 188 | 0.34 | 3.10 | 2.77 | 18.6 | 12.5 |
| DF | A1 | 54.5 | 3.5 | 428 | 1.20 | 3.74 | 3.61 | 23.2 | 15.8 |
| | A2 | 22.3 | 2.1 | 422 | 0.39 | 4.28 | 5.01 | 31.6 | 18.5 |
| | AB | 16.1 | 1.3 | 403 | 0.32 | 3.85 | 3.23 | 24.0 | 16.7 |
| | Bw | 9.9 | 1.5 | 423 | 0.48 | 3.91 | 3.81 | 27.5 | 17.6 |
| | CB | 7.0 | 0.8 | 332 | 0.70 | 3.87 | 2.99 | 23.0 | 16.9 |

### *3.4. Properties of aggregate associated-organic matter*

In the surface horizon (A1 horizon), the OC/N ratio was high (>14), in particular in T and DF sites; the amount of humic C (HC) was 13, 11 and 18 g $kg^{-1}$ for CS, T and DF sites, and the HC/OC percentage progressively increased from CS to DF (Figure 5).

Below A1 horizon, along the CS soil profile, the OC/N ratio decreased. Instead, along T and DF soil profile, aggregates from AB horizons were enriched in organic material characterised by higher OC/N ratio than that of the overlying A2 or underlying Bw horizons. Among sites, higher OC/N ratio values were generally observed in DF and T than CS soil, while HC/C ratio values were lower especially in A2 and AB horizons.

In order to better understand the main features of organic composition (both of total organic matter and humic fraction in aggregates), the IR spectra have been examined in the region between 1800-1300 $cm^{-1}$ (Figures 6 and 7).

The organic matter of aggregates from soil horizons (A1, A2, BA and CB) covered by Chestnut plants (CS) showed the most significant spectral profile modifications in the 1800-1300 $cm^{-1}$ studied region (Figure 6a). The horizon A1, as expected, was dominated by bands (1730, 1630, 1530 and 1378 $cm^{-1}$) indicating the presence of functional groups originated from litter decomposition. Moreover, Figure 6a shows the changes of the absorption of band related to H-O-H-bending band of water. The position of this band is gradually shifted from 1636 to 1624 $cm^{-1}$ along the horizons profile from A1 to CB horizon. A progressive shift in the bending frequency provides evidence that the amount of hydrogen bonded $H_2O$ molecules present in BA and CB is lower than those in A1 and A2 horizons. Probably the highest SOM amount found in A1 and A2 might favour the ability to form hydrogen bonds with adjacent $H_2O$ molecules.

An important change in organic matter properties was observed in the transition site (T) where Chestnut trees were replaced by conifer plants. However a part of Chestnut litter was also found in the area of conifer litter. Aggregates from A1 to Bw soil horizons showed in the region from 1800 to 1300 $cm^{-1}$ a broad band near 1630 $cm^{-1}$, a shoulder at 1528 $cm^{-1}$ and weak bands at 1454 and 1400 $cm^{-1}$ (Figure 6b). The band assigned to lignin (1528 $cm^{-1}$) was much higher in aggregates from A2 horizon than AB and Bw and up to disappear in CB horizon.

In addition, the water bending frequency showed slight shift in aggregates from A1 to Bw (from 1632 to 1636 $cm^{-1}$) whilst in aggregates from CB horizon a considerable shift (1614 $cm^{-1}$) was displayed.

The region of spectra from 1800 to 1300 $cm^{-1}$ of aggregates from A1, A2 and AB horizons covered by conifer plants (DF site) were very similar between them (Figure 6c). They differed for the band located at 1368 $cm^{-1}$ that disappeared in AB horizon. Bw and CB differed by the other horizons for the absence of the band due to aromatic rings (1530 $cm^{-1}$). The spectra of aggregates from deeper soil layers tended to be relatively homogenous in the studied sites.

The humic fraction spectra of three sites were very similar to corresponding total organic matter (Figure 7). Curve fitting in the region between 2900-1200 $cm^{-1}$ was performed to estimate how the relative peak areas changed to each other in relation to different aggregates and replaced vegetal species. The HC relative peak areas from different aggregates are shown in Figure 8.

With increasing the horizons depth, HC from site covered by Chestnut plants (CS) showed a decrease in relative intensity of aliphatic bands at 2922 $cm^{-1}$ while carboxylate groups (1600 $cm^{-1}$) remained constant until to BA and considerably decreased in CB horizon. Aromatic rings were more concentrated in BA horizon. Similarly to CS site, in the transition one (T) the carboxylate groups remained constant until to AB but then considerably increased in Bw reaching the relative

intensity values visible in DF site. In the deeper CB horizon of T site, the carboxylate again considerably decreased. Along the soil DF profile, the HC had extremely high relative intensity of carboxylate groups, which slightly decreased in AB and CB horizons.

The presence of aliphatic and aromatic groups appeared to be mainly related to the amount of humic C (r=0.692 and 0.635, respectively; Table 4). Instead, amorphous Al and Fe oxides was related to the relative intensity of carboxylate groups (r=0.715 and 0.697, respectively).

**Figure 5** – Organic C-to-N ratio (OC/N), humic compounds (HC) and percentage of HC to OC in the studied sites

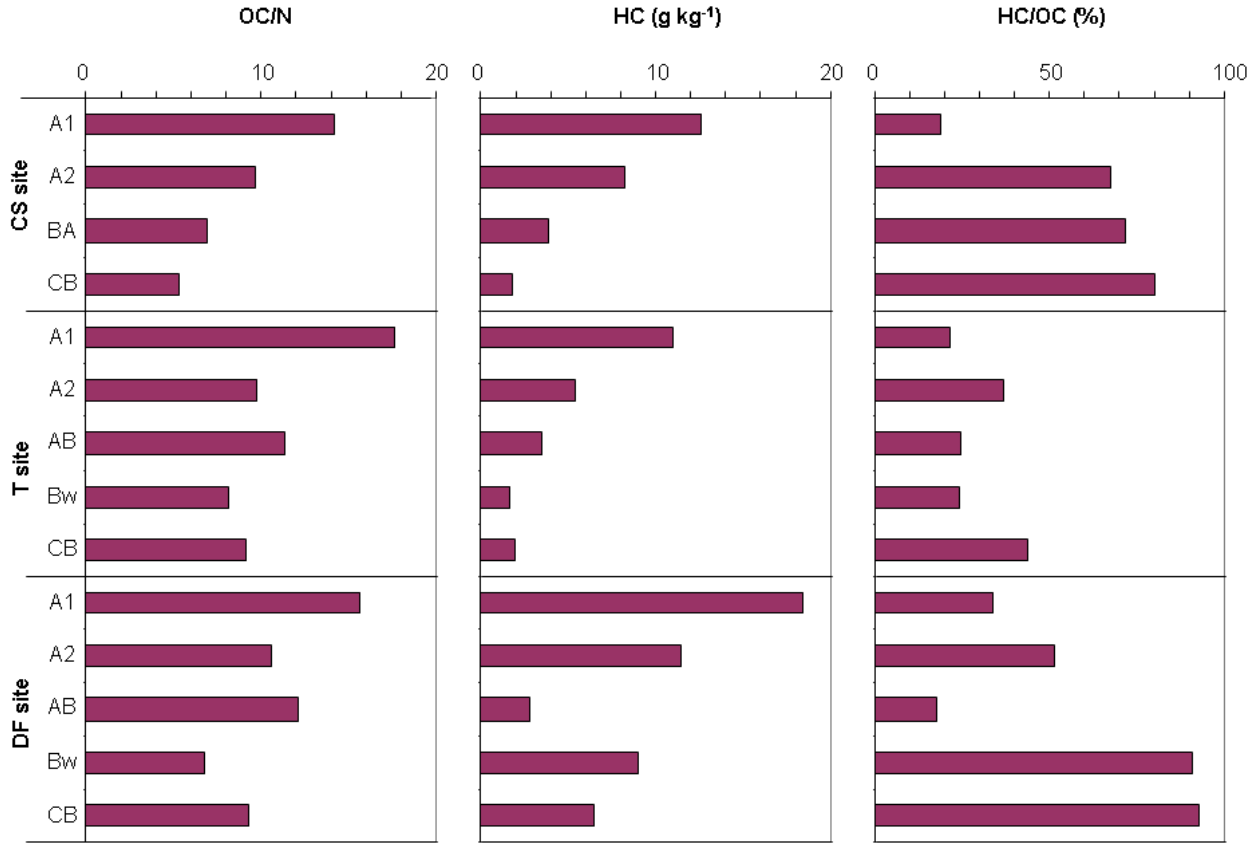


**Figure 6** – Attenuated total reflectance infrared Fourier transform (ATR/FT-IR) spectra of aggregates (AG) of the three soils: a) CS site; b) T site; c) DF site

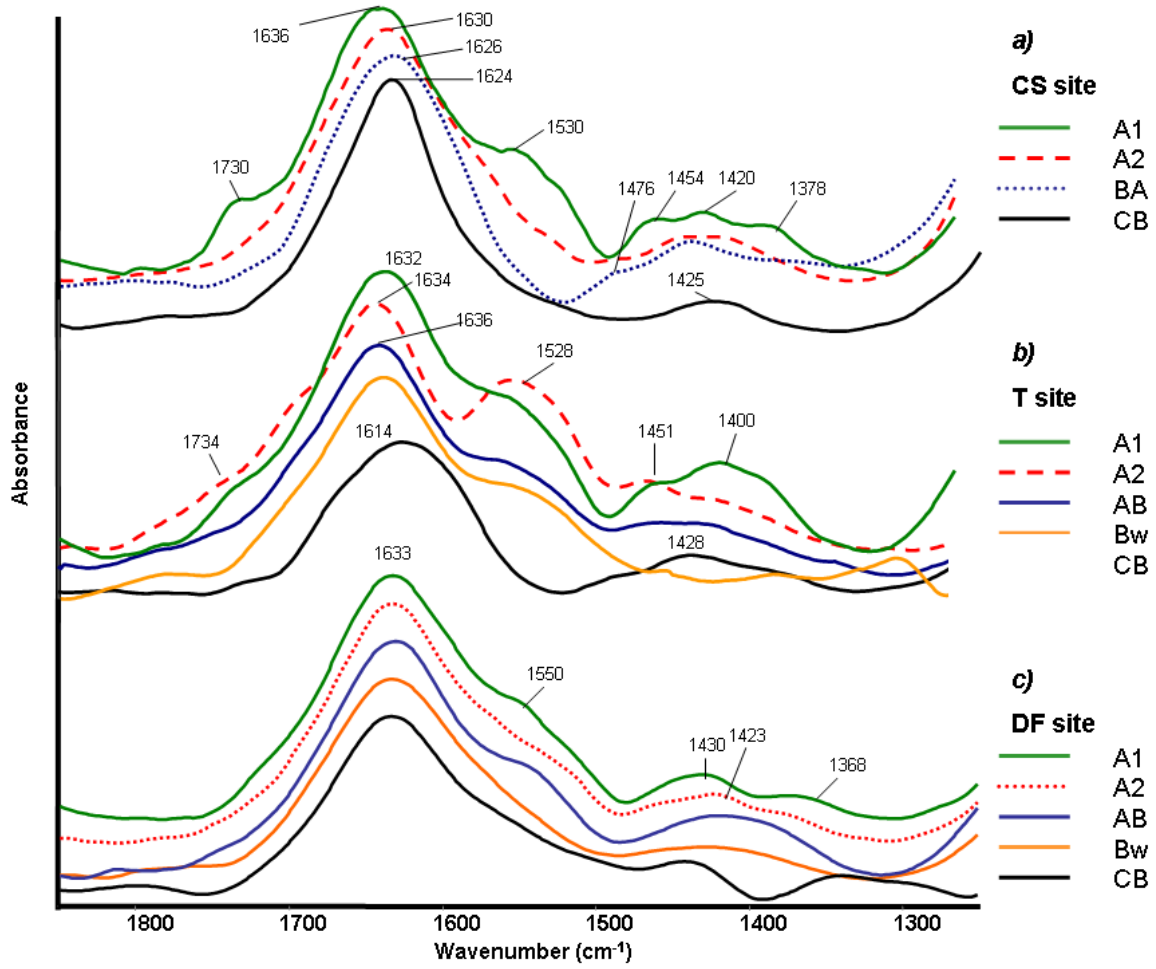


**Figure 7** – Attenuated total reflectance infrared Fourier transform (ATR/FT-IR) spectra of humic carbon (HC) extracted from aggregates (AG) of the three soils: a) CS site; b) T site; c) DF site

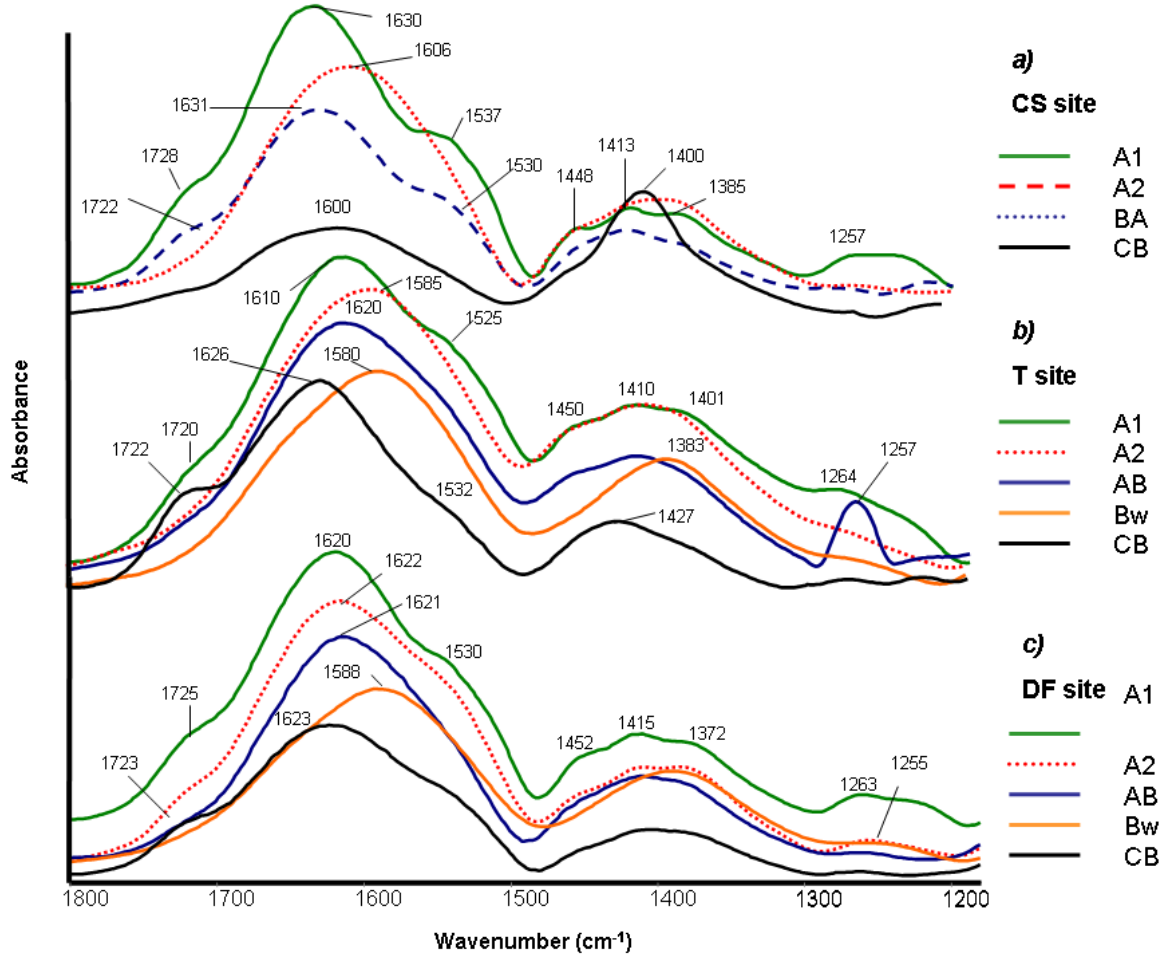


**Figure 8** – The humic carbon (HC) relative peak areas from the aggregates (AG)

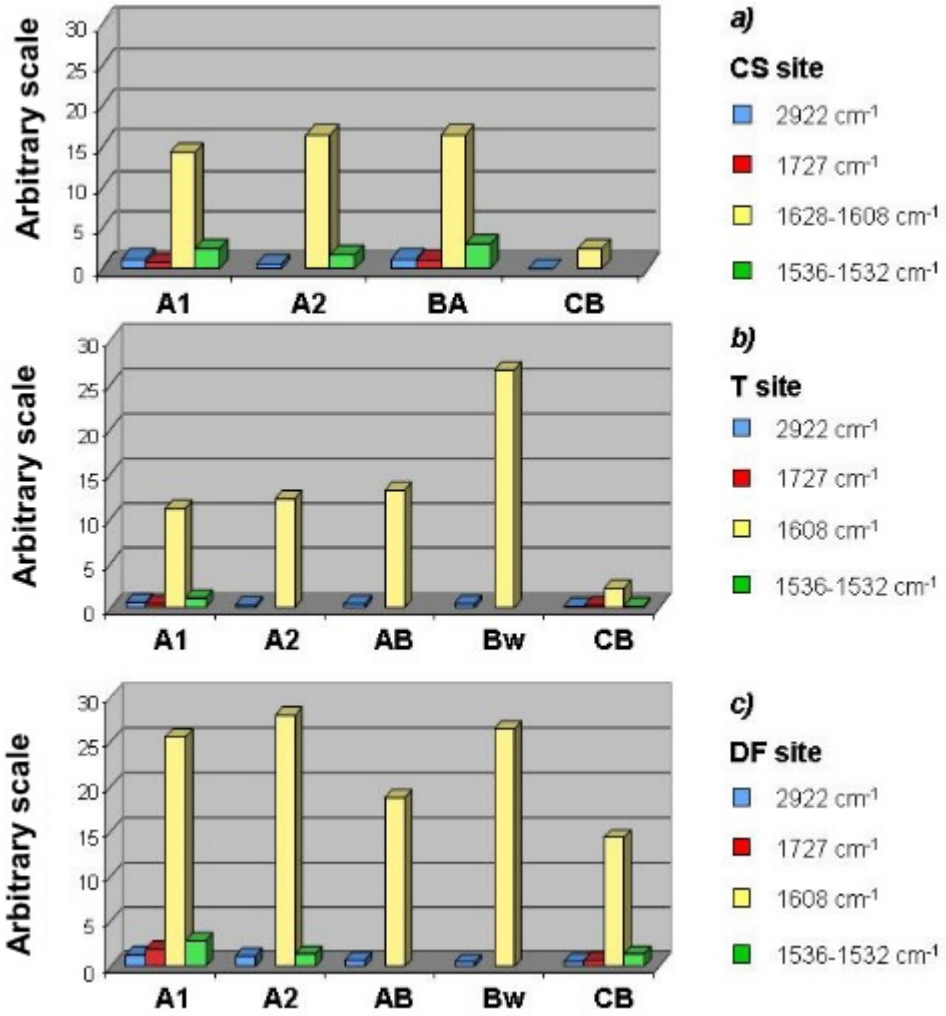


**Table 4** - Pearson's (r) correlation coefficients ~~of~~ between the intensities of bands attributed to aliphatic, carboxylate and aromatic groups of HC and aggregate properties

| | CH-Aliphatic | Carboxylate | Aromatic ring |
|---|---|---|---|
| pH | -0.449 | -0.149 | -0.359 |
| SOM | 0.598* | 0.165 | 0.519 |
| N | 0.654* | 0.276 | 0.525 |
| HC | 0.692** | 0.451 | 0.635* |
| clay | -0.286 | 0.007 | -0.263 |
| exchangeable-Ca | 0.431 | -0.006 | 0.551* |
| exchangeable-Mg | 0.577* | 0.111 | 0.564* |
| exchangeable-K | 0.620* | 0.145 | 0.597* |
| Alo | 0.247 | 0.715** | -0.200 |
| Feo | 0.280 | 0.697** | -0.014 |

*: $p<0.05$; **: $p<0.01$

### *3.5. Water aggregate stability*

When all mechanisms of aggregate breakdown were considered, thus both fast wetting and water abrasion acted in the destabilization of aggregates, the resistance of aggregates (i.e., $WAS_t$) decreased along the soil profile in all sites (Figure 9). When only water abrasion was taken into account, the aggregate stability ($WAS_{ab}$) decreased along the soil profile but in T and DF site the aggregates from A1 horizons had lower resistance than those from the underlying A2 horizons (Figure 9). However, both aggregate stability indexes were significant related to organic matter content ($r_s$=0.859 and 0.719, respectively for $WAS_t$ and $WAS_{ab}$, $p<0.01$; Figure 10), and significant linear relationships between water stability indexes and aggregate organic matter content were also found excluding A1 horizons (r=0.879 and 0.771, respectively for $WAS_t$ and $WAS_{ab}$, , $p<0.01$). With the exception of a very weak correlation with $WAS_t$, if A1 horizons were taken into account HC fraction was not related to the aggregate stability (Table 5), but its functional aliphatic and carboxylate groups were involved in the enhancement of aggregate resistance against abrasion.

**Figure 9** – Indexes of aggregate stability to water saturation and abrasion ($WAS_t$) and to water abrasion ($WAS_{ab}$)

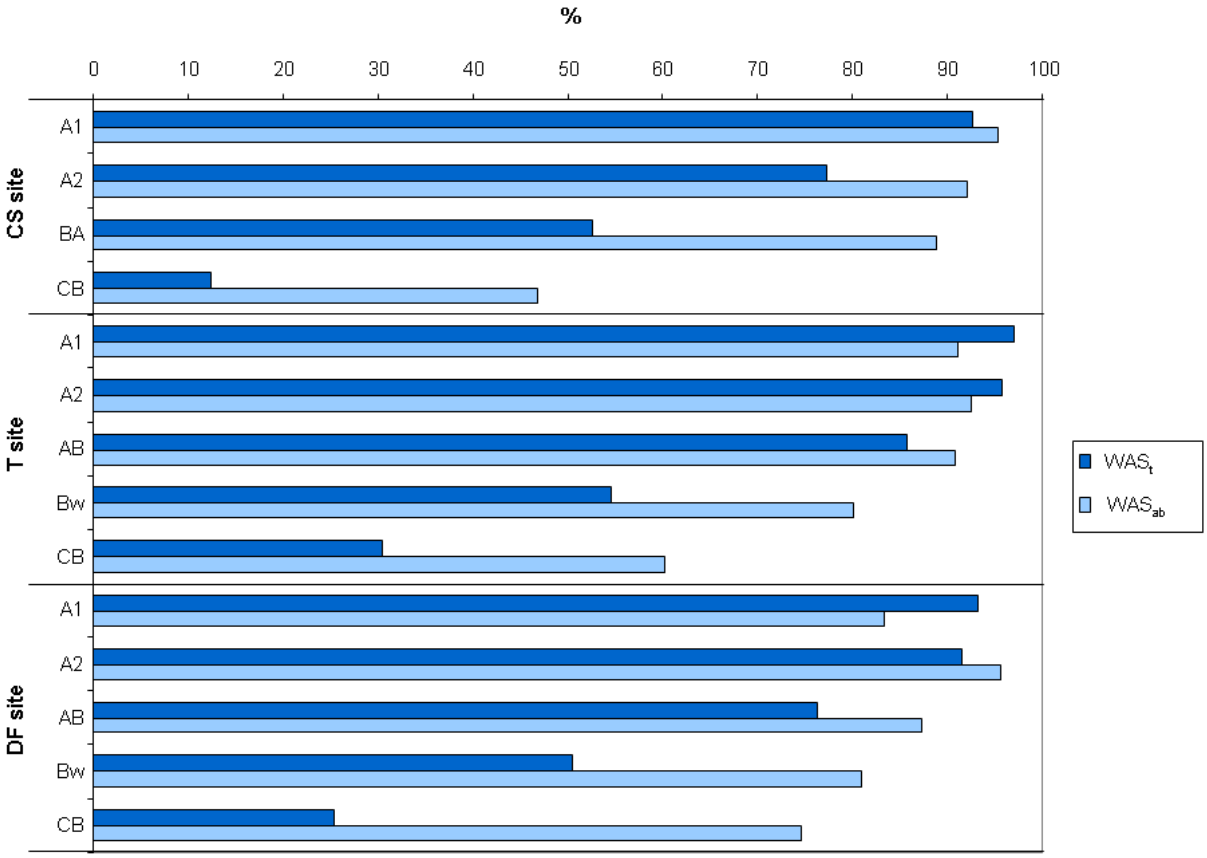



**Figure 10 -** Relationships between the amount of SOM and aggregate stability (a) to water saturation and abrasion ($WAS_t$) and (b) to water abrasion ($WAS_{ab}$)

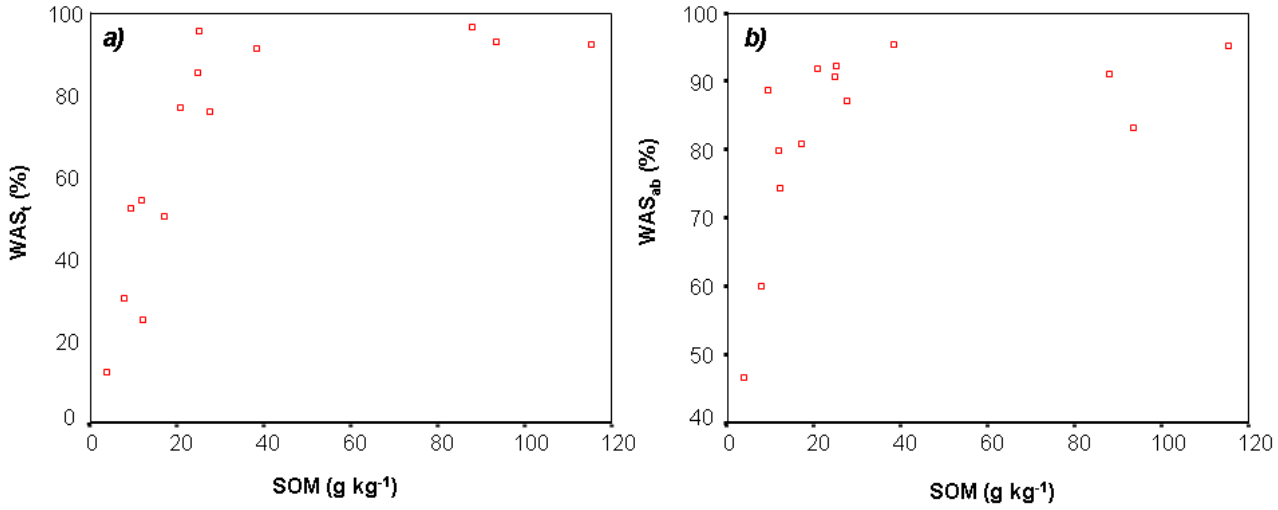


**Table 5** – Pearson's (r) correlation coefficients ~~of~~ between aggregate stability indexes and humic component characteristics

| all soil horizons | | | without A1 horizons | | |
|---|---|---|---|---|---|
| r | $WAS_t$ | $WAS_{ab}$ | r | $WAS_t$ | $WAS_{ab}$ |
| HC | 0.583* | 0.450 | HC | 0.404 | 0.523 |
| CH-Aliphatic | 0.529 | 0.625* | CH-Aliphatic | 0.425 | 0.680* |
| Carboxylate | 0.395 | 0.543* | Carboxylate | 0.472 | 0.659* |
| Aromatic ring | 0.264 | 0.366 | Aromatic ring | 0.004 | 0.324 |

$WAS_t$: water aggregate stability to saturation and abrasion; $WAS_{ab}$: water aggregate stability to abrasion; HC: carbon of humic component; *: $p<0.05$; **: $p<0.01$

### *3.6. Stable macroaggregate-OC and nutrients*

Total concentration of organic C and of other elements in the stable aggregates (MA) was reported in Table 6. In order to better understand the role of organic properties on loss or gain of element, we checked the relationships between the element content in MA and the amount of HC (Table 7). We found that the amount of HC was correlated to all the elements, and the most strong correlations were found with OC, N and Ca, which were the element that showed significant difference in the enrichment/depletion index among sites (Figure 11). In fact, the loss of OC progressively increased from CS to T and DF site, whereas N and Ca showed an opposite trend (Figure 11).

Also functional groups were related to the element content in MA, and aliphatic groups correlated with the element content in MA similarly to HC, but weaker relationships were found, whereas carboxylate groups had strong relationships especially with Al and Fe (Table 7). No relationships were instead found for aromatic groups.

**Table 6** – Total element concentration of the water stable aggregates (MA).

| Site | Horizons | OC | N | $P_t$ | $Ca_t$ | $Mg_t$ | $K_t$ | $Al_t$ | $Fe_t$ |
|---|---|---|---|---|---|---|---|---|---|
| | | g $kg^{-1}$ | | mg $kg^{-1}$ | g $kg^{-1}$ | | | | |
| CS | A1 | 71.2 | 4.7 | 600 | 1.15 | 3.16 | 3.22 | 20.7 | 15.3 |
| | A2 | 11.4 | 1.0 | 220 | 0.44 | 2.80 | 2.54 | 17.3 | 13.6 |
| | AB | 6.4 | 0.6 | 134 | 0.32 | 2.43 | 2.37 | 15.3 | 11.8 |
| | CB | 2.0 | 0.3 | 71 | 0.53 | 1.83 | 2.39 | 11.1 | 9.1 |
| T | A1 | 59.6 | 3.6 | 275 | 1.03 | 2.50 | 2.92 | 17.1 | 12.2 |
| | A2 | 13.8 | 1.3 | 216 | 0.38 | 2.94 | 2.80 | 18.1 | 14.1 |
| | AB | 15.2 | 1.6 | 294 | 0.28 | 2.92 | 2.73 | 19.4 | 14.3 |
| | Bw | 5.9 | 1.1 | 261 | 0.33 | 2.31 | 2.37 | 15.6 | 12.3 |
| | CB | 2.9 | 0.5 | 129 | 0.38 | 1.55 | 2.12 | 11.0 | 7.3 |
| DF | A1 | 52.7 | 3.7 | 400 | 1.20 | 3.34 | 3.80 | 21.9 | 15.3 |
| | A2 | 22.8 | 2.5 | 416 | 0.36 | 3.87 | 4.75 | 27.5 | 17.3 |
| | AB | 13.2 | 1.7 | 392 | 0.38 | 3.57 | 3.50 | 22.3 | 16.5 |
| | Bw | 8.6 | 1.5 | 370 | 0.40 | 3.08 | 2.99 | 20.3 | 15.6 |
| | CB | 4.8 | 0.8 | 223 | 0.35 | 1.99 | 2.01 | 13.1 | 10.9 |

**Table 7** – Pearson's (r) correlation coefficients between the amount of element in water stable aggregate (MA) and humic component characteristics

| r | OC | N | $P_t$ | $Ca_t$ | $Mg_t$ | $K_t$ | $Al_t$ | $Fe_t$ |
|---|---|---|---|---|---|---|---|---|
| HC | 0.776** | 0.806** | 0.644* | 0.767** | 0.549* | 0.622* | 0.583* | 0.527 |
| aliphatic | 0.573* | 0.643* | 0.629* | 0.457 | 0.634* | 0.624* | 0.650* | 0.604* |
| carboxylate | 0.126 | 0.311 | 0.558* | 0.015 | 0.689** | 0.589* | 0.718** | 0.748** |
| aromatic | 0.508 | 0.458 | 0.272 | 0.521 | 0.200 | 0.223 | 0.201 | 0.165 |

HC: carbon of humic component; *: $p<0.05$; **: $p<0.01$

## 4. Discussion and Conclusions

In the studied Chestnut-Douglas fir biosequence, the content and chemical composition of aggregate associated-organic C were influence by plant species.

Under Chestnut plants, the aggregate associated-organic matter showed the most significant spectral profile modifications. In fact, the hemicellulose persistance (1730 and 1378 $cm^{-1}$) might

suggest that it is partly integrated with lignin and therefore, is not more available and hydrolysed by microorganisms. On the contrast, the lack of lignin aromatic rings in macroaggregates from A2 to CB soil horizons may indicate that lignin decomposition was occurred. These changes are in accordance with different SOM content and OC/N ratio found (Table 1 and Figure 5, respectively).

In the transition site (T) where Chestnut trees were replaced by conifer plants, but a part of Chestnut litter was also found in the area of conifer litter, the ATR/FT-IR spectra showed a change in the intensity of the band assigned to lignin (1528 $cm^{-1}$) from A2 horizon to deeper ones. This result might mean that aggregates from A2 horizon preferentially retain aromatic molecules because the decomposition of needle-leaf are slower than from Chestnut leaf trees. Usually needle-leaf contains a mixture of acidic and neutral diterpenes and together with a variable fraction of volatile components (monoterpenes and sesquiterpenes; Langenheim, 2003) that are important in the defence systems of conifers. These products could slow down the microbial activity and, in turn, the humification process with respect to other tree leaf, as confirmed by the sharply decrease of HC/OC percentage (Figure 5). Moreover, topsoils affected by Douglas fir plants (i.e., T and DF site) had the highest OC/N, further confirming that tree species are the most important explanatory variable for the OC/N also in aggregate associated-organic matter as already observed for the bulk soil (Cools et al., 2014). High OC/N ratio had an inhibitory effect of microbial activity similar to retention of aromatic compounds. In fact as the humification process proceeds, the C/N ratio decreases and the organic matter becomes more resistant to hydrolysis. These chemical changes generally varies with depth and the organic matter shows a progressive loss of carbohydrates, hydroxyl and methoxyl groups and, to a lesser extent, lignin, and an increase in carboxyl and carbonyl groups (Kögel-Knabner et al., 1991).

Under Douglas fir plants, apparently aggregates had lesser lignin content than transition site where aromatic rings were dominant in deeper soil horizons.

These results mean, therefore, that the vegetation has a pronounced effect on soil properties and that broad-leaf trees could give more favourable condition for aggregate associated-organic carbon formation than needle-leaf trees (van Breemen and Finzi, 1998).

However, both under Chestnut and Douglas fir, the organic matter was the main aggregating agents, even if we observed that the less transformed organic residues in the topsoils had low efficiency in the formation of aggregates. In these top soils, the difference between $WAS_t$ and $WAS_{ab}$ values suggested that under Douglas fir aggregates had high water repellence probably due the presence of hydrophobic compounds, and enhanced aggregate stability (e.g., Bachmann et al., 2008). Therefore, even if the less transformed organic matter in top soils was less active in

aggregate formation than that of the deeper horizons, under Douglas fir contributed to aggregate stability more efficiently than under Chestnut trees.

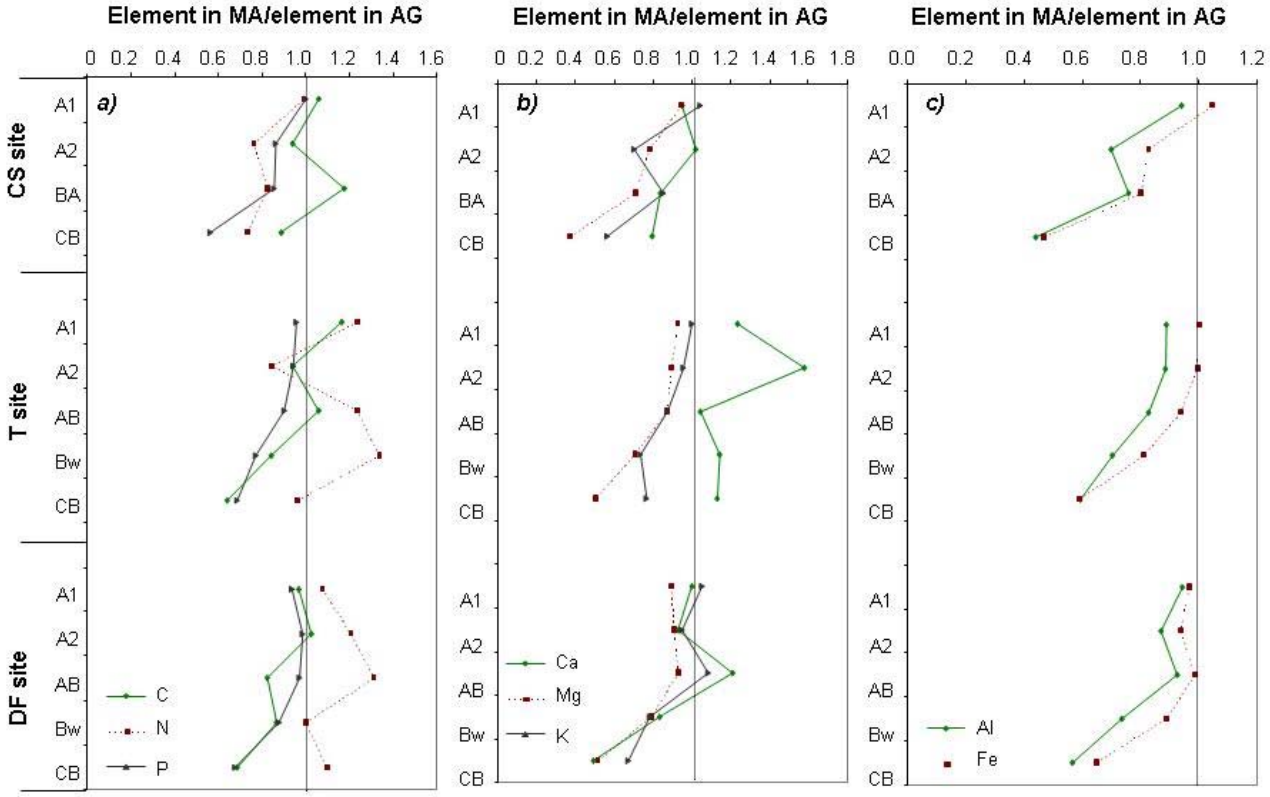
In the deeper horizons (i.e., underlying A1 horizons) the organic matter was again the main cementing agents and the amount of water stable aggregates linearly increased according to the content of organic compounds. Organic matter also affected aggregates stability by interaction with metal, in fact the amount of stable aggregates increased as amorphous Al (i.e., humus-Al compounds) increased. Thus, organic matter influenced aggregate stability both directly through transient or more persistent organic cements and indirectly by formation of stable organo-metal complexes (e.g., Tisdall and Oaedes, 1998; Six et al., 2004; Bronick and Lal, 2005). Other common cementing agents, such as clay and exchangeable Ca, appeared instead more involved in the formation of microaggregates released by aggregate breakdown, as their amounts negatively correlated with MA.

The different chemical properties of aggregate associate-organic matter seemed to affect the relative importance of direct and indirect mechanisms (organic cements and organic-metallic cements, respectively) that, as consequence, influenced the aggregate stability and the loss of OC and of other nutrients. In fact, our results showed that under Douglas fir plants, high presence of carboxylate groups favour the complexes formation with metals (Al and Fe). On one side, the organo-metal interaction stabilised organic matter, as it is well known (e.g., von Lützow et al., 2006), and on the other side stabilised aggregates (e.g., Bronick and Lal, 2005) in particular against water abrasion, as our results showed.

This indirect mechanism seemed to less efficiently limit the C loss from aggregates, at least in the studied 1-2 mm size aggregates. This was evident from the progressive increase of loss of C from DF to CS sites. Under Douglas fir, the retained organic compounds in the stable macroaggregates were N-richer than under Chestnut trees, and the macroaggregates selectively retained more transformed organic compounds (i.e., lower OC/N ratio) that interact with metal as previously discussed. Interactions between organic matter and other elements, such as Ca, also prevent C losses in DF e T soils, as lower Ca depletions were detected than in CS site. However, a more deeper investigation needs to better understand the involvement of Ca in organic complexes.

Under Chestnut trees, organic matter direct actions prevalently occurred and fresh, more C-rich organic materials were preserved. In CS site lower losses of C occurred from water stable macroaggregates than in DF or T site, suggesting that in our study case direct mechanism was more efficient in C stabilisation in aggregate that indirect mechanism.

**Figure 11 – E**nrichment and depletion in element of water stable aggregate (MA) respect to the 1-2 mm aggregates (AG): (a) C, N and P; (b) Ca, Mg and K; (c) Al and Fe. Value higher than 1 indicates an element enrichment.



# Chapter IV: Bio-climate factors, soil aggregates stability and C preservation: the case study of Corno alle Scale mountain (Northern Appennine)

## 1. Introduction

Understanding the fate of soil organic matter (SOM) has become especially important in the global climate change, because SOM has a key role in many biogeochemical processes in soil, and it responses rapidly to environmental changes.

Vegetation type and elevation are major controlling factors for net primary production. These factors also act on the storage and turnover of the organic compounds in the soil. As reviewed by Stockmann et al. (2013), soil organic C sink capacity of world soils differs among biomes (see also Chapter 1) and Jobbágy and Jackson (2000) stressed the role of vegetation type on SOM content, especially in the C distribution with depth in the soil; while temperature has been often taken into account in altitudinal gradients of SOM dynamics (Kutsch et al., 2009) because it acts as a limiting factor for microbial activity at higher altitudes. Conant et al. (2011) proposed a conceptual model defining the resistance of soil OM to decomposition as due either to the depolymerization process or to the adsorprtion/desoption and aggregate turnover; they discussed the former as a temperature dependent process, while a more complex temperature response was hypothesize for the latter. Schmidt et al. (2011) stated that the persistence of SOM is primarily not a molecular property, but an ecosystem property; in other words, the SOM persists in the soil not because of the intrinsic properties of the organic compounds itself, but because of physicochemical and biological influences from the surrounding environment that reduce the rate of OM decomposition, thereby allowing its accumulation in the soil. Soil aggregates are the main component of soil, and they have significant effects on soil biophysical properties. The physical protection of soil organic C by stable aggregate is an important mechanisms of soil carbon preservation (Lal, 2013) and, as also stressed by von Lützow et al. (2006), its relevance increases in the topsoil horizons.

As observed by Gunina and Kuzyakov (2014) C stabilization in aggregates lead to $^{13}C$ fractionation due to both to preferential stabilization of organic compounds with light or heavy $\delta^{13}C$ and/or to stabilization of organic materials after passing one or more microbial utilization cycles, leading to heavier $\delta^{13}C$ in remaining C. They suggest that new $^{13}C$ natural abundance approach is suitable for analysis of C pathways by SOM transformation, and in our opinion this approach could

be suitable to study C pathways by SOM segregation in stable aggregates from soils of different phtytoclimatic ecosystems.

The aims of this study were thus i) to evaluate the capacity in topsoils and subsoils to preserve C in aggregates stable to different water stress (i.e., fast wetting and fast wetting plus water abrasion) and ii) to verify if any variation in site factors, such as vegetation and elevation, affected stabilisation of aggregates and, thus, aggregate capacity of C preservation. In this study we also took into account the relationships between C preserved into aggregates and other elements (such as N, P, S, Ca and also Al, Fe, Mg and K) and applied $\delta^{13}C$ isotopic ratio to characterise the organic C preserved into water stable aggregates. Because of mountain area offers steep environmental gradients representing natural physicochemical and biological conditions (Körner, 2007) characterising several phytoclimatic ecosystems, we chosen the Corno alle Scale mountain as study area. Corno alle Scale mountain is in fact one of the highest peak in the northern Italian Appennines on Cevarola massive sandstone, it offers a variety of habitats that makes it a suitable area for our evaluations in a large suite of phytoclimatic ecosystems typical of mountain area.

## 2. Materials and Methods

### *2.1. The study area*

Corno alle Scale mountain is located in the northern Emilian-Tuscany Appenines (44°07'07"N, 10°49'47"E, Italy) and reaches 1944 m of height. Meadows alternate with coniferous forests (mainly *Abies alba,* Miller) cover most of the *Picetum* zone (above 1500 m asl according to the Pavari's phytoclimatic classification; De Philippis, 1951), while beech forests (*Fagus sylvatica,* L.) and Douglas fir (*Pseudotsuga menziesii* Franco) reforestations dominate the *Fagetum* phytoclimatic zone (below 1500 m asl). Among the *Fagetum* and *Picetum* zone, there is a strong gradient of temperature with mean air temperature of 9°C and 5°, respectively, while the annual precipitation varied from 1300 to 3200 mm. Soil develop on Cervarola massive sandstone, with particle size from coarse to fine, consisting in a prevailing silica-clastic components such as quartz, feldspar, mica, biotite and chlorite.

Along the phytoclimatic gradient, nine sites in similar and comparable topographic position and representative of vegetation cover were selected from 1000 to 1800 m (Table 1). Beech forest and Douglas fir reforestation dominate in the *Fagetum* zone, and three sites in beech forest (Corn 7, 9 and 10) and two in Douglas fir reforestation were chosen (Corn 6 and 8). In the *Picetum* zone, a large variability in the vegetation cover type was present, and thus one site was selected in a Silver

fir forest (Corn 3), one site in a natural beech forest at its higher climatic limit of growth (Corn 4), two sites in meadows at different altitude, where grasses and bilberry (*Vaccinium myrtillus,* L.) dominate at the lowest and highest site, respectively (Corn 1 and 12). In the study area, the elevation limit between the phytoclimatic *Fagetum* and *Picetum* zone marks the pedoclimatic transition between mesic and frigid soil termperature regime. All soils were ascribed to Cambisols, with the exception of Corn 10 and Corn 1 that were ascribed to Regosols and Leptsols, respectively (IUSS, ISRIC, FAO 2006).

At each site, a representative soil profile was selected (Table 1). In order to study the effect of vegetation factor co-varying with the climate factors, soil samples were taken from solum, thus from the upper part of soil profile (A, B, but not C horizons) in which the soil-forming processes predominantly occur (Chesworth, 2008). For each soil profiles, soil properties of each studied horizon were then averaged and grouped as topsoil (A horizons) and subsoil (B, AC or AB horizons). Thus, in this study the reported results were referred to topsoil and subsoil horizons.

**Table 1 –**General information on soils

| Elevation | Plant cover | Site | Studied genetic horizons | Lower limit of horizons[§] |
|---|---|---|---|---|
| m asl | | | | cm |
| 1009 | Beech forest (BF) | CORN9 | A1, A2, A3, Bw | 3, 8, 12, 28 |
| 1038 | Douglas fir reforestation (DR) | CORN8 | A, Bw | 13, 27 |
| 1231 | Douglas fir reforestation (DR) | CORN6 | A, Bw1, Bw2 | 8, 21, 35 |
| 1254 | Beech forest (BF) | CORN7 | A, AB, Bw | 3, 15, 41 |
| 1424 | Beech forest (BF) | CORN10 | A1, A2, AB | 2, 7, 15 |
| 1566 | Grassland – non forested (NF) | CORN1 | A, AC | 9, 11 |
| 1607 | Beech forest (BF) | CORN4 | A1, A2, Bw | 4, 6, 10 |
| 1680 | Silver fir forest (SF) | CORN3 | AB, Bw1, 2Bw2 | 3, 15, 25 |
| 1788 | Vaccinetum – non forested (NF) | CORN12 | A1, A2, Bw | 5, 13, 36 |

*2.2. Macroaggregate fractionation*

The bulk soil samples collected from each genetic mineral horizon were air-dried to constant weight and dry-sieved to pass a 2-mm screen (<2 mm). Then, the 1-2 mm aggregates (AG) were separated from the fine earth fraction by dry sieving to 1 mm and weighted. A weighted aliquot of AG was placed in 0.2-mm sieve and wetted by immersion in water for 10 min (i.e., water saturation), then wet sieved at 60 cycles $min^{-1}$ (i.e., water abrasion). At the end of wet sieving, the aggregates remaining in the sieves were oven dried at 40°C, weighted and their amount was expressed as the percentage of AG ($MA_t$). This separated fraction represented the macroaggregates stable to water stress (i.e., water saturation and abrasion). Another aliquot of AG was placed in 0.2-

mm sieve and wetted by immersion in water for 10 min (i.e., water saturation) and the aggregates remaining in the sieves were oven dried at 40°C, weighted and their amount was expressed as the percentage of AG ($MA_{fw}$). This separated fraction represented the macroaggregates stable to fast wetting stress (i.e., water saturation).

*2.3. Measurement of the 1-2 mm macroaggregate stability to the different breakdown mechanisms*

The wet stability of the 1-2 mm aggregates to different aggregate breakdown mechanism was determined by Yoder's (1936) wet sieving procedure (Kemper and Rosenau, 1986) using pretreatments as a way to elucidate the mechanisms and processes involved in aggregate breakdown (Hénin et al., 1958; Le Bissonnais, 1990).

The aggregate stability to water saturation and abrasion ($WAS_t$) was determined as follows

$$WAS_t = \frac{MA_t - coarse\,sand}{100 - corse\,sand} \cdot 100$$

$WAS_t$, MA and coarse sand were expressed as percentage. The coarse sand fraction (>0.2-mm) was determined on 1-2 mm aggregate after $H_2O_2$ oxidation.

The aggregate stability to water saturation alone ($WAS_{fw}$) was determined as

$$WAS_{fw} = \frac{MA_{fw} - coarse\,sand}{100 - corse\,sand} \cdot 100$$

$WAS_{fw}$, $MA_{fw}$ and coarse sand were expressed as percentage. The coarse sand fraction (>0.2-mm) was determined on 1-2 mm aggregate after $H_2O_2$ oxidation.

*2.4. Main physico-chemical properties of macroaggregates (AG)*

The 1-2 mm aggregates were characterised for the main physico-chemical properties involved in soil aggregation and nutrient status (see also Chapter 3). The pH was determined potentiometrically in a 1:2.5 (w:v) soil-deionised water suspension for mineral or organic horizons (Van Reeuwijk, 2002). The texture was obtained by the pipette method after dispersion of the sample with a sodium hexametaphosphate solution (Gee and Bauder 1986). The total organic carbon (TOC) and total nitrogen (N) contents were measured by dry combustion (EA-1110 Thermo Scientific Lab). The exchangeable cations ($Ca^{2+}$, $Mg^{2+}$, $Na^{+}$, $K^{+}$) were determined by Inductively Coupled Plasma Optical emission Spectrometry (ICP-OES, Amtek, Arcos Spectro) after exchange with 1M $NH_4^{+}$-acetate at pH 7 (Summer and Miller, 1996).

Both the interaction of $Al^{3+}$ and $Fe^{3+}$ with SOC, as organo-metallic compounds, and oxides-hydroxides of Fe can promote aggregation in acidic soils (e.g., Six et al., 2004). Therefore, the amount of both amorphous Al and Fe forms and well-crystalline pedogenic Al and Fe oxides was determinated. Amorphous iron (Feo) and aluminium (Alo) forms were estimated through extraction with acid-ammonium oxalate (Schwertmann, 1964), and Fe and Al in the extracts were measured by ICP-OES. Pedogenic iron oxides (Fed) were estimated through extraction with Na–dithionite–citrate–bicarbonate (Mehra and Jackson, 1960) and Fe in the extracts was analysed by ICP-OES. The total element concentrations ($Al_t$, $Fe_t$, $Ca_t$, $Mg_t$, $K_t$, $Na_t$, $P_t$ and $S_t$) were measured by ICP-OES after $HNO_3$:HCl (1:3) microwave-digestion of sample.

*2.5. Elements concentration of water stable macroaggregates ($MA_t$ and $MA_{fw}$)*

The total organic carbon (TOC) and total nitrogen (N) contents of $MA_t$ and $MA_{fw}$ were measured by dry combustion, while the concentration of Al, Fe, Ca, Mg, K, Na, P and S were measure by ICP-OES after $HNO_3$:HCl (1:3) microwave-digestion of sample as described above. They represent the element in the macroaggregates stable to water saturation and abrasion and to water saturation alone, respectively.

*2.6. $\delta^{13}C$ analysis*

The stable C isotopic ratios of fractioned macroaggregates (soil macroaggregates – AG, stable macroaggregates to water saturation and abrasion - $MA_t$, and stable macroaggregates to water saturation – $MA_{fw}$) were measured by using IRMS coupled with an elemental analyser (EA-1110 Thermo Scientific Lab).

The isotopic compostion of the samples is expressed as $\delta^{13}C$ notation (‰) relative to the international standard Vienna-Pee Dee Belemite (VPDB):

$$\delta^{13}C‰ = \frac{R_{sample} - R_{stdVPDB}}{Rs_{tdVPDB}}$$

where $R={}^{13}C/{}^{12}C$.

*2.7. Statistical analysis*

All statistical analyses were carried out using SPSS software package (SPSS Inc., Chicago, IL). The correlations were estimated using Pearson's coefficients. A probability level of 0.05 was always used as threshold for significance.

## 3. Results and Discussion

### *3.1. Soil aggregate distribution and aggregates properties*

The amount of 1-2 mm aggregates (AG) varied from 14 to 36% in the top horizons (Figure 1A) of which 58-87% was stable to fast wetting and water abrasion ($MA_t$). The amount of aggregates stable to fast wetting ($MA_{fw}$) varied from 81 to 97%. As expected, the amount of $MA_{fw}$ was higher than that of $MA_t$, because only the aggregate breakdown due to water saturation was considered. The mechanical breakdown due to water abrasion further produced aggregate rupture obviously decreasing the amount of stable aggregates (Le Bissonnais, 1996).

Similarly to the top horizons, in the subsoil the percentage of AG ranged from 18 and 38 (Figure 1B). Instead, the amount of $MA_t$ and $MA_{fw}$ were slightly lower ranging between 54-83 and 66-93%, respectively. This is in agreement with the data reported for other mountain soils. In fact, deeper horizons have often lower resistance to the aggregate breakdown and therefore lower amount of stable aggregates. This is due to the positive effect of organic matter on the stabilization of aggregates, as well documented (e.g., Bronick and Lal, 2005). The improvement of aggregate stability due to higher amount of SOM in the topsoils than subsoil has been observed even at low contents of organic C. For example, Castro Filho et al. (2002), who compared soil surface and bottom layers, found a higher aggregate stability in the topsoil even if C was only about 10 g $kg^{-1}$.

In the studied aggregates, the pH was acidic (Table 2). The concentration of soil organic matter decreased from the top to the subsoil in all sites varying, respectively, from 61.1 to 157.2 and from 18.4 to 50.4 g $kg^{-1}$ (Table 2). This data supports our previous hypothesis, and that was the decreasing contents of organic C through the soil profile that caused decreasing in the amount of MA, because a lower aggregation capacity occurred in subsoil due to the lower SOM content.

The effect of SOM on aggregate formation was evident also in smaller aggregated-particles, such as coarse sand-sized particles (from 0.2 to 2 mm of size). In this study, the coarse sand fraction corresponded to the largest particles size and, because we determined it after chemical dispersion without any removal of organic matter cements, the coarse sand fraction represented aggregated-particles. Coarse sand fraction was significant related to the content of SOM ($r=0.613$; $p=0.009$) confirming thus the role of organic cements in the aggregation processes in these soils. As consequence, in our soils the amount of clay fraction (i.e., non-aggregated finer particle) increased with depth.

The SOM affected also the amount of exchangeable cations, as visible by the decreasing of exchangeable Ca, Mg and K with depth in all site (Table 2).

Instead, the amount of pedogenic Al and Fe oxides (Alo, Feo, Fed; Table 2) generally increased with depth, as well as the Fed/Fet ratio. Data of Al or Fe fractionation referred to the fine earth fraction is commonly used in the assessment of soil weathering processes (e.g., Arduino et al., 1986; D'Amico et al., 2008). In our studied aggregates, both the oxide contents and the Fed/Fet ratio had quite high values (on the average 1.7, 6.9 and 15.6 g $kg^{-1}$, and 0.59, respectively for Alo, Feo, Fed and Fed/Fet). For example, in the fine earth fraction of similar mountain soils, the Fed/Fet ratio ranged from 0.17 to 0.28 (Vittori Antisari et al., 2011). It seems therefore that an accumulation of pedogenic Fe oxides had occurred in the aggregates, suggesting that also pedogenic Al and Fe oxides should play a role in the aggregates formation. As well known, Al and Fe improve soil aggregation acting as bridging agents (e.g., Amezketa, 1999; Bronick and Lal, 2005; see also Chapter 1). Therefore, in our studied soils an involvement of SOM and pedogenic Al or Fe oxides in aggregate stability is expected.

In Table 3, the total Al, Fe, Ca, Mg, K, P and S concentration of soil aggregates was reported. As visible, Al and Fe had the highest concentration while Ca had low concentration confirming the mineral composition of the Cervarola massive.

**Figure 1** – Distribution of percentage of 1-2 mm aggregates (AG), macroaggregates stable (MA) to water saturation and abrasion and to water abrasion ($MA_{fw}$) in the studied sites: A) topsoils; B) subsoils

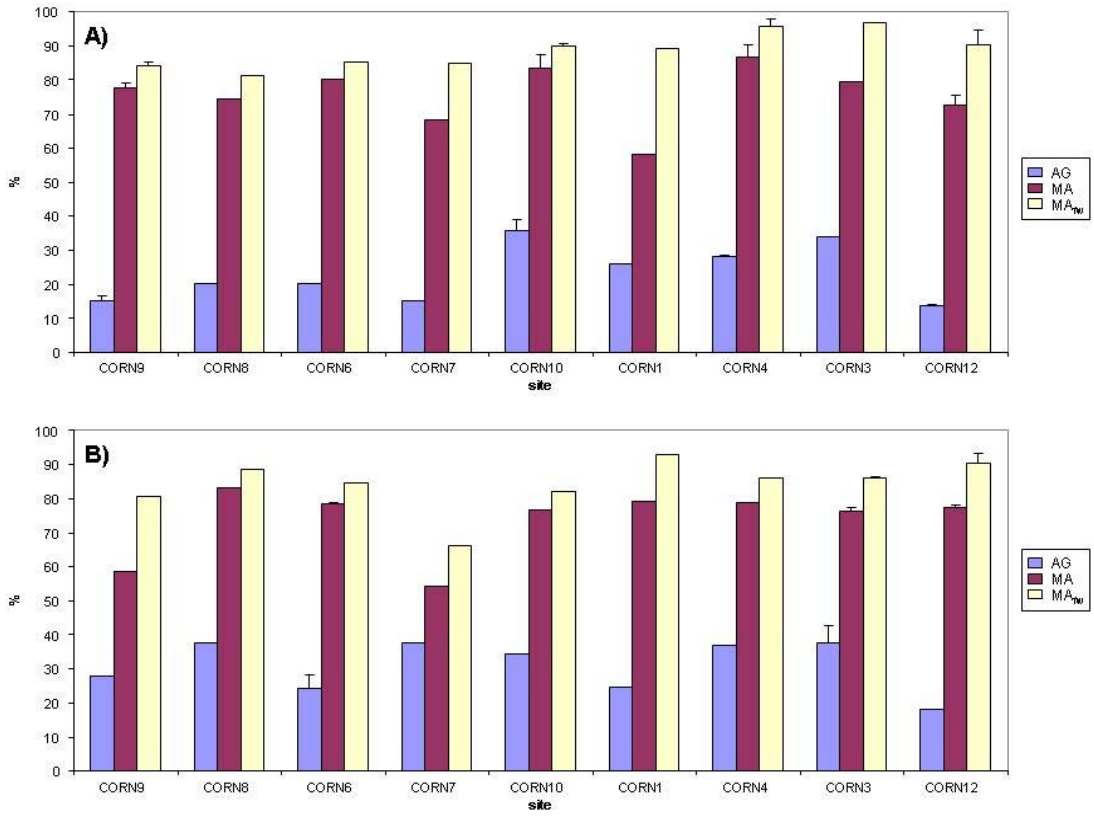


**Table 2 –** Main physico-chemical properties of the 1-2 mm aggregates

| Elevation (m asl) - Plant cover | Site | Horizons | pH | SOM | N | $sand_C$ | clay | $Ca_{exch}$ | $Mg_{exch}$ | $K_{exch}$ [§] | Alo | Feo | Fed | Fed/Fet |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | g $kg^{-1}$ | | | | $cmol_+kg^{-1}$ | | | g $kg^{-1}$ | | | |
| 1009 – BF | CORN9 | topsoil | 6.4 | 68.2 | 2.2 | 655 | 17 | 14.34 | 1.35 | 0.32 | 0.42 | 2.17 | 8.19 | 0.37 |
| | | subsoil | 5.9 | 27.8 | 0.9 | 352 | 34 | 1.31 | 0.15 | 0.09 | 0.70 | 2.56 | 10.62 | 0.50 |
| 1038 – DR | CORN8 | topsoil | 5.7 | 61.1 | 3.1 | 568 | 74 | 19.00 | 2.18 | 0.65 | 1.20 | 4.41 | 13.29 | 0.46 |
| | | subsoil | 4.5 | 38.3 | 1.9 | 190 | 181 | 1.50 | 0.49 | 0.15 | 1.20 | 4.91 | 15.64 | 0.55 |
| 1231 – DR | CORN6 | topsoil | 4.5 | 87.9 | 3.5 | 673 | 2 | 0.19 | 0.07 | 0.04 | 1.31 | 3.25 | 8.27 | 0.42 |
| | | subsoil | 4.9 | 38.8 | 1.7 | 587 | 56 | 0.10 | 0.03 | 0.04 | 2.34 | 4.08 | 9.84 | 0.49 |
| 1254 – BF | CORN7 | topsoil | 5.1 | 116.6 | 4.2 | 692 | 13 | 1.99 | 0.49 | 0.20 | 1.21 | 6.28 | 11.86 | 0.58 |
| | | subsoil | 5.1 | 18.4 | 1.0 | 301 | 108 | 0.18 | 0.05 | 0.04 | 2.85 | 9.44 | 15.79 | 6.66 |
| 1424 – BF | CORN10 | topsoil | 4.8 | 81.8 | 4.3 | 637 | 46 | 1.84 | 0.43 | 0.09 | 1.63 | 8.44 | 16.47 | 0.69 |
| | | subsoil | 5.0 | 41.3 | 2.3 | 359 | 85 | 0.32 | 0.11 | 0.04 | 2.17 | 11.19 | 19.60 | 0.78 |
| 1566 - NF | CORN1 | topsoil | 5.4 | 105.7 | 5.8 | 628 | 20 | 13.73 | 1.33 | 0.32 | 0.43 | 3.11 | 7.38 | 0.33 |
| | | subsoil | 5.5 | 26.0 | 1.8 | 64.5 | 35 | 4.52 | 0.20 | 0.08 | 0.21 | 2.42 | 14.51 | 0.42 |
| 1607 – BF | CORN4 | topsoil | 4.3 | 157.2 | 5.0 | 617 | 109 | 1.20 | 0.45 | 0.74 | 1.83 | 6.82 | 17.43 | 0.64 |
| | | subsoil | 4.9 | 35.6 | 1.6 | 398 | 243 | 0.55 | 0.28 | 0.11 | 2.37 | 4.79 | 12.50 | 0.33 |
| 1680 – SF | CORN3 | topsoil | 3.8 | 126.0 | 6.3 | 472 | 98 | 0.25 | 0.16 | 0.06 | 1.62 | 13.82 | 26.55 | 0.99 |
| | | subsoil | 4.2 | 50.4 | 2.9 | 265 | 124 | 0.08 | 0.06 | 0.04 | 2.08 | 12.54 | 24.81 | 0.84 |
| 1788 - NF | CORN12 | topsoil | 4.8 | 69.4 | 3.8 | 656 | 33 | 0.39 | 0.16 | 0.05 | 2.52 | 9.44 | 21.24 | 0.69 |
| | | subsoil | 4.6 | 19.0 | 2.0 | 448 | 58 | 0.12 | 0.04 | 0.03 | 2.73 | 10.72 | 26.61 | 0.77 |

SOM: soil organic matter determined by multiply the content of organic C by 1.72; $sand_C$: coarse sand (2-0.2 mm); [§] amount of exchangeable-Na was always <0.01 $cmol_+$ $kg^{-1}$

**Table 3** – Total Al, Fe, Ca, Mg, K, P and S concentration of soil aggregates

| Elevation (m asl) - Plant cover | Site | Horizons | Al | Fe | Ca | Mg | K | P | S |
|---|---|---|---|---|---|---|---|---|---|
| | | | g kg$^{-1}$ | | | | | mg kg$^{-1}$ | |
| 1009 - BF | CORN9 | topsoil | 33.9 | 22.1 | 2.6 | 11.4 | 6.7 | 286 | 191 |
| | | subsoil | 31.3 | 21.1 | 1.9 | 10.3 | 5.4 | 204 | 83 |
| 1038 - DR | CORN8 | topsoil | 45.1 | 28.9 | 1.2 | 13.1 | 9.2 | 427 | 257 |
| | | subsoil | 34.3 | 28.7 | 0.6 | 12.0 | 5.2 | 361 | 182 |
| 1231 - DR | CORN6 | topsoil | 29.4 | 19.6 | 1.1 | 7.5 | 5.4 | 604 | 343 |
| | | subsoil | 32.0 | 20.2 | 0.6 | 8.5 | 6.2 | 518 | 222 |
| 1254 - BF | CORN7 | topsoil | 31.8 | 20.6 | 9.9 | 10.5 | 8.3 | 595 | 951 |
| | | subsoil | 35.5 | 23.7 | 17.0 | 10.1 | 10.9 | 311 | 79 |
| 1424 - BF | CORN10 | topsoil | 34.7 | 23.7 | 1.5 | 7.0 | 7.9 | 1061 | 429 |
| | | subsoil | 36.4 | 25.1 | 0.7 | 6.2 | 7.9 | 937 | 284 |
| 1566 - NF | CORN1 | topsoil | 33.9 | 22.7 | 3.0 | 11.5 | 8.8 | 636 | 474 |
| | | subsoil | 41.1 | 28.1 | 2.1 | 14.3 | 10.9 | 450 | 162 |
| 1607 - BF | CORN4 | topsoil | 36.1 | 27.6 | 1.5 | 7.1 | 8.1 | 546 | 416 |
| | | subsoil | 44.0 | 37.4 | 0.4 | 8.9 | 10.1 | 389 | 187 |
| 1680 - SF | CORN3 | topsoil | 39.4 | 24.6 | 0.5 | 5.2 | 9.8 | 1063 | 638 |
| | | subsoil | 34.5 | 29.5 | 0.3 | 5.6 | 8.3 | 779 | 305 |
| 1788 - NF | CORN12 | topsoil | 51.4 | 30.0 | 1.2 | 8.4 | 11.8 | 567 | 365 |
| | | subsoil | 62.7 | 34.6 | 0.5 | 9.5 | 14.1 | 427 | 176 |

*3.2. Isotopic composition and TOC:N ratio in the aggregates*

The $\delta^{13}C$ values in aggregates (AG) of top horizons varied from -26.82 to -25.22‰. Subsoil horizons had lower values that varied from -26.22 to -25.18‰ and an enrichment in $^{13}C$ natural abundance was always observed with respect to the corresponding top horizon (i.e., higher values in subsoils; Table 4). The value of total organic C-to-N ratio (TOC/N) varied from 5.6 to 18.1 and, similarly to the $\delta^{13}C$ values, it showed a trend with depth, generally decreasing from the top horizons to the corresponding subsoils (Table 4).

To explain the shift of $\delta^{13}C$ values with soil depth, some authors suggested that a role could be played by microbial discrimination toward the heaviest isotope during respiration, which would leave the soil enriched with $^{13}C$ isotopes (Šantrůčková et al., 2000); other hypotheses included

a greater incorporation of humified C compared to non-decomposed C and a preservation of recalcitrant organic material with a low isotopic ratio (Devevre and Horwath, 2001). For our aggregates, we checked the relationships between $\delta^{13}C$ values and the other aggregates properties

and we found that the TOC/N ratio, which is a common indicator of the soil organic matter turnover (Bronick and Lal, 2005), was significant negatively related (r=-0.617, p=0.006) to the $\delta^{13}C$ values. Also the amount of amorphous Fe forms (i.e., Feo) was significant positively related to the $\delta^{13}C$ values (r=0.660, p=0.004). These relationships indicated that the $^{13}C$ isotopes abundance increased with reduction of the degree of transformation (higher TOC/N ratio) of organic compounds and with increase of the interaction with Fe. Thus, they would confirm that the organic matter in the subsoil horizons was likely both more decomposed and transformed by microbial process and more preserved by metal complexation or interaction with minerals than in top horizons.

**Table 4** - The $\delta^{13}C$ values and total organic C-to-N (TOC/N) ratio of soil aggregates

| Elevation (m asl) - Plant cover | Site | Horizons | TOC/N | $\delta^{13}C$ |
|---|---|---|---|---|
| | | | | ‰ |
| 1009 – BF | CORN9 | topsoil | 17.2 | -26.64 |
| | | subsoil | 17.8 | -25.67 |
| 1038 – DR | CORN8 | topsoil | 11.4 | -26.69 |
| | | subsoil | 11.7 | -25.27 |
| 1231 – DR | CORN6 | topsoil | 14.8 | -26.82 |
| | | subsoil | 13.3 | -25.89 |
| 1254 – BF | CORN7 | topsoil | 16.3 | -26.82 |
| | | subsoil | 10.2 | -25.50 |
| 1424 – BF | CORN10 | topsoil | 11.0 | -25.71 |
| | | subsoil | 10.4 | -25.47 |
| 1566 – NF | CORN1 | topsoil | 10.7 | -26.23 |
| | | subsoil | 8.5 | -25.43 |
| 1607 – BF | CORN4 | topsoil | 18.1 | -26.66 |
| | | subsoil | 12.6 | -26.22 |
| 1680 – SF | CORN3 | topsoil | 11.6 | -25.22 |
| | | subsoil | 10.2 | -25.18 |
| 1788 - NF | CORN12 | topsoil | 10.2 | -25.60 |
| | | subsoil | 5.6 | -25.51 |

### *3.3. Variation in aggregates properties due to aggregates breakdown*

The element concentrations in the macroaggregates stable to breakdown ($MA_t$ and $MA_{fw}$) were compared to those in the corresponding aggregates (AG) and enrichement/depletion indexes (i.e., element concentration in MA/element concentration in AG) were reported in Tables 5 and 6.

**Table 5** – Enrichment/depletion index in total organic C, N, Al, Fe, Ca, Mg, K, P and S concentration of macroaggregates stable to fast wetting ($MA_{fw}$) and macroaggregates stable to fast wetting and water abrasion ($MA_t$) in the top horizons. Value higher than 1 indicates an element enrichment

| Elevation (m asl) - Plant cover | Site | | TOC | N | Al | Fe | Ca | Mg | K | P | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1009 - BF | CORN9 | $MA_{fw}$ | 0.92 | 0.91 | 1.11 | 1.05 | 0.82 | 0.96 | 1.08 | 0.95 | 0.90 |
| 1038 - DR | CORN8 | $MA_{fw}$ | 0.91 | 0.96 | 1.07 | 1.04 | 0.61 | 0.93 | 0.95 | 0.87 | 0.73 |
| 1231 - DR | CORN6 | $MA_{fw}$ | 0.93 | 0.92 | 1.14 | 1.06 | 0.99 | 1.03 | 1.21 | 0.96 | 1.01 |
| 1254 - BF | CORN7 | $MA_{fw}$ | 1.00 | 0.98 | 1.16 | 1.12 | 1.01 | 1.05 | 1.13 | 1.03 | 1.15 |
| 1424 - BF | CORN10 | $MA_{fw}$ | 1.07 | 1.12 | 1.15 | 1.11 | 1.00 | 1.02 | 1.13 | 1.04 | 1.11 |
| 1566 - NF | CORN1 | $MA_{fw}$ | 1.02 | 1.00 | 1.32 | 1.19 | 1.26 | 1.18 | 1.31 | 1.21 | 1.22 |
| 1607 - BF | CORN4 | $MA_{fw}$ | 1.04 | 1.07 | 1.10 | 1.06 | 0.99 | 1.04 | 1.08 | 1.05 | 1.04 |
| 1680 - SF | CORN3 | $MA_{fw}$ | 1.02 | 0.98 | 1.02 | 1.11 | 1.34 | 1.09 | 0.99 | 1.05 | 1.06 |
| 1788 - NF | CORN12 | $MA_{fw}$ | 0.98 | 1.00 | 1.18 | 1.13 | 0.60 | 1.09 | 1.11 | 1.08 | 1.11 |
| | | *mean* | *0.99* | *0.99* | *1.14* | *1.10* | *0.96* | *1.04* | *1.11* | *1.03* | *1.03* |
| | | *min* | *0.92* | *0.91* | *1.02* | *1.04* | *0.60* | *0.93* | *0.95* | *0.87* | *0.73* |
| | | *max* | *1.07* | *1.12* | *1.32* | *1.19* | *1.34* | *1.18* | *1.31* | *1.21* | *1.22* |
| 1009 - BF | CORN9 | $MA_t$ | 1.04 | 0.98 | 1.07 | 0.99 | 0.84 | 0.90 | 1.04 | 0.96 | 1.01 |
| 1038 - DR | CORN8 | $MA_t$ | 0.96 | 0.95 | 1.03 | 0.99 | 0.99 | 0.91 | 0.94 | 0.95 | 0.98 |
| 1231 - DR | CORN6 | $MA_t$ | 0.87 | 0.93 | 1.14 | 1.06 | 0.97 | 1.02 | 1.26 | 0.95 | 0.97 |
| 1254 - BF | CORN7 | $MA_t$ | 0.93 | 1.02 | 1.05 | 1.06 | 1.00 | 0.97 | 1.02 | 0.93 | 0.87 |
| 1424 - BF | CORN10 | $MA_t$ | 1.02 | 1.07 | 1.15 | 1.02 | 0.93 | 0.98 | 1.12 | 0.97 | 1.03 |
| 1566 - NF | CORN1 | $MA_t$ | 0.84 | 0.92 | 1.35 | 1.23 | 1.05 | 1.19 | 1.34 | 1.11 | 1.04 |
| 1607 - BF | CORN4 | $MA_t$ | 1.36 | 1.34 | 1.10 | 1.00 | 1.33 | 0.97 | 1.08 | 1.18 | 1.28 |
| 1680 - SF | CORN3 | $MA_t$ | 1.05 | 1.06 | 1.13 | 1.04 | 1.21 | 1.13 | 1.11 | 1.10 | 1.17 |
| 1788 - NF | CORN12 | $MA_t$ | 0.88 | 1.08 | 1.10 | 1.04 | 0.57 | 1.01 | 1.07 | 0.96 | 1.03 |
| | | *mean* | *1.01* | *1.04* | *1.12* | *1.05* | *0.99* | *1.01* | *1.11* | *1.01* | *1.04* |
| | | *min* | *0.84* | *0.92* | *1.03* | *0.99* | *0.57* | *0.90* | *0.94* | *0.93* | *0.87* |
| | | *max* | *1.36* | *1.34* | *1.35* | *1.23* | *1.33* | *1.19* | *1.34* | *1.18* | *1.28* |

**Table 6** – Enrichment/depletion index in total organic C, N, Al, Fe, Ca, Mg, K, P and S concentration of macroaggregates stable to fast wetting ($MA_{fw}$) and macroaggregates stable to fast wetting and water abrasion ($MA_t$) in the subsoils. Value higher than 1 indicates an element enrichment

| Elevation (m asl) - Plant cover | Site | | TOC | N | Al | Fe | Ca | Mg | K | P | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1009 - BF | CORN9 | $MA_{fw}$ | 0.93 | 0.99 | 1.23 | 1.09 | 0.87 | 1.03 | 1.33 | 1.01 | 0.99 |
| 1038 - DR | CORN8 | $MA_{fw}$ | 1.00 | 1.03 | 1.32 | 1.01 | 2.09 | 0.99 | 1.54 | 1.10 | 1.29 |
| 1231 - DR | CORN6 | $MA_{fw}$ | 0.98 | 0.99 | 1.19 | 1.12 | 0.87 | 0.98 | 1.21 | 1.00 | 0.89 |
| 1254 - BF | CORN7 | $MA_{fw}$ | 1.00 | 1.03 | 1.25 | 1.13 | 0.19 | 1.30 | 0.89 | 1.09 | 1.20 |
| 1424 - BF | CORN10 | $MA_{fw}$ | 1.05 | 1.05 | 1.18 | 1.08 | 0.91 | 1.00 | 1.16 | 1.05 | 1.08 |
| 1566 - NF | CORN1 | $MA_{fw}$ | 1.16 | 1.07 | 1.16 | 1.13 | 1.12 | 1.09 | 1.13 | 1.08 | 6.91 |
| 1607 - BF | CORN4 | $MA_{fw}$ | 1.14 | 1.08 | 1.11 | 1.06 | 1.10 | 1.04 | 1.07 | 1.05 | 1.05 |
| 1680 - SF | CORN3 | $MA_{fw}$ | 1.04 | 1.02 | 1.19 | 1.08 | 1.28 | 1.10 | 1.18 | 1.06 | 1.05 |
| 1788 - NF | CORN12 | $MA_{fw}$ | 0.94 | 0.97 | 1.04 | 1.09 | 0.81 | 1.04 | 0.99 | 0.94 | 0.97 |
| | | *mean* | *1.03* | *1.02* | *1.19* | *1.09* | *1.03* | *1.06* | *1.17* | *1.04* | *1.71* |
| | | *min* | *0.93* | *0.97* | *1.04* | *1.01* | *0.19* | *0.98* | *0.89* | *0.94* | *0.89* |
| | | *max* | *1.16* | *1.08* | *1.32* | *1.13* | *2.09* | *1.30* | *1.54* | *1.10* | *6.91* |
| 1009 - BF | CORN9 | $MA_t$ | 1.05 | 1.12 | 1.23 | 1.06 | 0.83 | 0.99 | 1.39 | 0.99 | 0.98 |
| 1038 - DR | CORN8 | $MA_t$ | 0.86 | 1.00 | 1.45 | 1.04 | 1.08 | 1.01 | 1.84 | 1.00 | 1.01 |
| 1231 - DR | CORN6 | $MA_t$ | 0.77 | 0.86 | 1.04 | 1.10 | 0.85 | 0.95 | 1.01 | 0.91 | 0.80 |
| 1254 - BF | CORN7 | $MA_t$ | 0.84 | 0.81 | 1.22 | 1.03 | 0.20 | 1.24 | 0.97 | 0.89 | 0.83 |
| 1424 - BF | CORN10 | $MA_t$ | 1.04 | 1.14 | 1.11 | 1.04 | 1.09 | 0.95 | 1.10 | 0.98 | 1.05 |
| 1566 - NF | CORN1 | $MA_t$ | 0.93 | 1.06 | 1.14 | 1.09 | 0.98 | 1.09 | 1.11 | 1.00 | 0.96 |
| 1607 - BF | CORN4 | $MA_t$ | 1.03 | 1.05 | 1.22 | 1.08 | 1.08 | 1.08 | 1.21 | 1.06 | 1.06 |
| 1680 - SF | CORN3 | $MA_t$ | 1.02 | 1.04 | 1.18 | 1.14 | 1.00 | 1.11 | 1.17 | 1.07 | 1.03 |
| 1788 - NF | CORN12 | $MA_t$ | 0.77 | 0.84 | 1.14 | 1.07 | 0.67 | 1.07 | 1.11 | 0.93 | 0.87 |
| | | *mean* | *0.92* | *0.99* | *1.19* | *1.07* | *0.84* | *1.05* | *1.21* | *0.98* | *0.95* |
| | | *min* | *0.77* | *0.81* | *1.04* | *1.03* | *0.20* | *0.95* | *0.97* | *0.89* | *0.80* |
| | | *max* | *1.05* | *1.14* | *1.45* | *1.14* | *1.09* | *1.24* | *1.84* | *1.07* | *1.06* |

In the topsoils, TOC content in the MA showed some variation with respect to that in the corresponding AG (Table 5). These variations seemed to be related to site elevation, vegetation and soil depth depth of soil. In fact, in the topsoils of the site under beech forest (BF) an enrichment in organic C in stable macroaggregates was generally observed along the elevation. Instead, the $\delta^{13}C$ values (Figure 2) seemed to increase from AG to $MA_t$ in the lower sites (i.e., CORN9 and CORN7) while decreased from AG to $MA_{fw}$ and $MA_t$ in the higher site (i.e., CORN 10 and 4). The shift of $\delta^{13}C$ suggested that $^{13}C$ was accumulated in the macroaggregates progressively more resistant to the different mechanisms of aggregate breakdown in the sites at lower elevation, but that the $^{13}C$ enrichment of stable macroaggregates decreased in the sites at higher elevation. This redistribution of C from aggregates (AG) to stable macroaggregates (MA) with elevation should suggested that differently transformed or mineral-associated organic matter formed stable macroaggregates according to the elevation. It appeared therefore that under beech forest at lower elevation the formation of stable macroaggregates occurred thought the accumulation of transformed organic compounds (i.e., shift in $\delta^{13}C$) probably interacting with mineral phases. This should correspond to the formation of microaggregates from organic molecules attached to mineral particles by polyvalent cationic binding to form stable macroaggregates, according to the hierarchical theory of aggregation (Edwards and Bremner, 1967; Tisdall, 1996; see also Chapter 1). Instead, at higher elevation, the formation of stable macroaggregates was ascribable to the presence of low transformed, more fresh and $^{13}C$ depleted organic matter. Thus, this should correspond to an alternative model of soil aggregation whereby stable macroaggregates can form around particulate organic matter (POM) partially decomposed (Six et al., 2004; see also Chapter 1).

The occurrence of the two model of aggregation could be due to a decline of microbial activity at higher site because the worsening of pedoclimatic condition. As described in the Materials and Methods section, the soils under beech had frigid and mesic soil temperature regime at higher and lower elevation, respectively. This mean that mean annual soil temperature of sites at higher elevation was between 0 and 8°C, while the soil temperature of sites at lower elevation was between 8 and 15°C (Soil Survey Staff, 2010). Decrease of mineralization rates of organic matter or topsoils in frigid or colder soil temperature regimes due to the inhibition of microbial activity is often reported (Schaetzl and Anderson, 2005).

In subsoil, no TOC enrichment trend was found along elevation, however a shift toward lower $\delta^{13}C$ values in MA than in AG was still visible in CORN10 (i.e., soil under beech cover at higher elevation; Figure 3). Even if smaller, the shift toward slightly lower values of $\delta^{13}C$ at higher site suggested that aggregates can form by similar aggregation processes than that of topsoils.

In non-forest sites (i.e., CORN1 and 12) in the topsoil the macroaggregates stable to fast wetting and water abrasion ($MA_t$) were the most C-depleted (Table 5), and, similarly to sites under beech, MA in frigid soil temperature regime were $^{13}C$-depleted (Figure 2). In subsoils, however, different trends were visible as a shift toward higher $\delta^{13}C$ value was found in CORN12 (Figure 3). The subsoil of CORN12 corresponded to a Bw horizon, contrasting to the AC horizon of CORN1 (Table 1). Moreover, the subsoil of CORN12 was more acidic and richer in pedogenic Al and Fe oxides than subsoil of CORN1 (Table 2). Thus, both morphological and chemical data pointed to an more intense weathering process in CORN12 than CORN1. This could be quite expected, because even if both soils developed under non-forest vegetation cover, CORN12 was under vaccinetum, while CORN1 under grassland. Thus, in our case, the more intense weathering process in CORN12 should allow a higher interaction between more transformed organic matter and mineral phase, and a higher involvement of organo-mineral complexes in the formation of stable aggregates than in the topsoil (Bronick and Lal, 2005).

No clear trend was found under coniferous (CORN8, 6 and 3) either in C amount or $^{13}C$ abundance. In CORN8 and 6 (i.e., under Douglas fir reforestation at lower elevation) the stable macroaggregates were always C-depleted with respect AG (Table 5 and 6). Conversely, in CORN3 (i.e., under Silver fir forest at higher elevation) the stable macroaggregates were always C-richer than AG (Table 5 and 6). As regards to $\delta^{13}C$ values, we observed than the organic matter from Silver fir was $^{13}C$ richer than that from Douglas fir especially in the topsoil. However, both under Douglas fir and Silver fir changes in $\delta^{13}C$ values from AG to MA were minimal, suggesting that in these sites no segregation of differently transformed or $^{13}C$ enriched organic materials occurred or was detectable on the basis of aggregate stability.

As regards other elements concentration, such as N, Al, Fe, Ca, Mg, K, P and S, both enrichment and depletion were observed in stable macroaggregates (Tables 5 and 6). The N, P and S in the stable macroaggregates followed the same trend than C. For Al and Fe an enrichment of MA was always observed with respected to the AG. Mg, K and Ca had a variable trend, but among theses elements Ca showed the highest depletion.

In order to identify the relationships between aggregate stability, preservation of C and of other elements, correlations between aggregate stability and elements indexes (i.e., enrichment/depletion indexes) were checked for both mechanisms and both soil layers.

For the fast wetting, the aggregate stability index ($WAS_{fw}$) was not correlated to any enrichment/depletion indexes both in top and subsoils. Instead, when all mechanisms acting in the

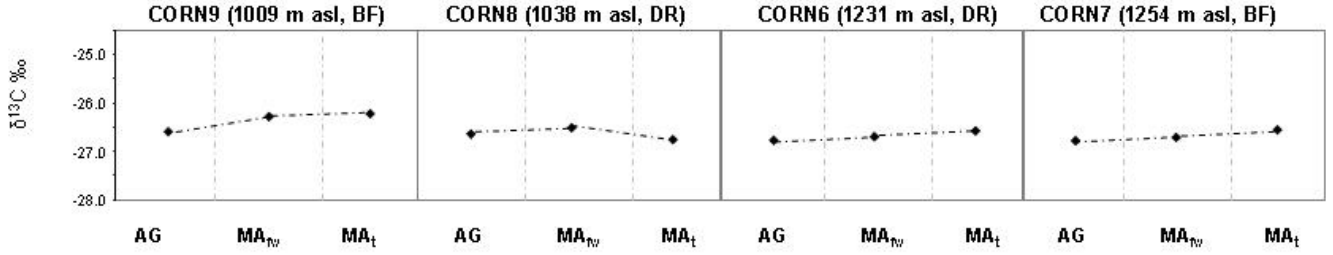
destabilization of aggregates were considered together, and thus both fast wetting and abrasion were was taken into account, some relationships were found (Table 7).

In topsoils the aggregate stability index ($WAS_t$) was positively related ($r=0.744$, $p<0.05$) to the TOC index (Table 7). Therefore, an increase in the stability of aggregates enhanced the preservation of organic C. This result stresses the mutual relationship between SOM and soil aggregate structure (Blanco-Canqui and Lal, 2004): SOM is a factor responsible for aggregates development, but aggregates exert an effect on SOM dynamics through the retention of organic C. The significant positive relationship between TOC index and $\delta^{13}C$ value ($r=0.64$, $p<0.05$; Table 7) further added information about the quality of preserved organic C, suggesting that in topsoils the most $^{13}C$-rich organic compounds were selectively preserved in stable aggregate. The correlations between the TOC index and those of N and S ($r>0.80$, $p<0.01$; Table 7), S and P ($r=0.88$, $p<0.01$), P and Ca ($r=0.73$, $p<0.05$) were of a certain interest, pointing to the role of organic compounds in the control of fate of the other macronutrients.

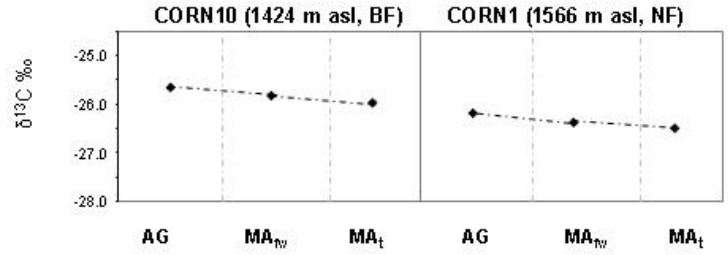
In topsoils, a significant relationship was also found between $WAS_t$ and Fe, but in this case the aggregate stability was inversely related to Fe index ($r=-0.77$, $p<0.05$). Moreover, Fe index was related to those of Al, Mg and K (r always $>0.80$, $r>0.01$). On one side these results could confirmed that aggregate stability contributes to preferentially preserve compounds of organic origin, on the other side it suggested a major role of compounds of lithogenic origin in microaggragates formation.

In subsoil $WAS_t$ was not related to TOC index (Table 7) in agreement to von Lützow et al. (2006), which stressed on the major relevance of physical protection of soil organic C by stable macroaggregate in the topsoil horizons than subsoils. However, even in the deeper horizons similar relationships to those in surface horizons were found for TOC index and $\delta^{13}C$ value, and N, P, S and Ca; also Fe index was again related to Al, Mg and K (Table 7). This data confirmed that also in subsoil, when TOC enrichment occurred in stable aggregate, a more $^{13}C$-rich organic compounds were preserved with an enhancement of compounds of organic origin and a corresponding depletion of lithogenic elements.

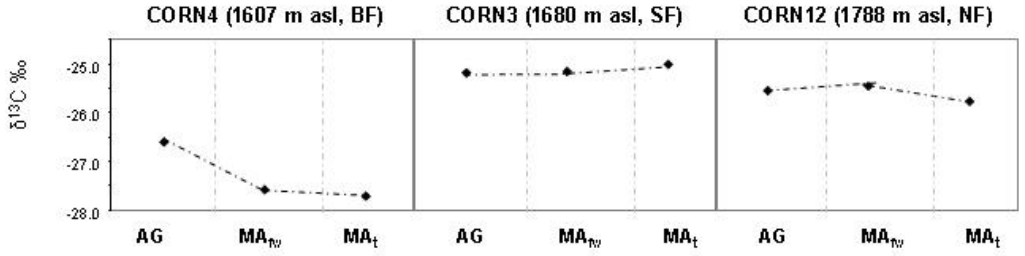
**Figure 2** – Water aggregate stability indexes ($WAS_t$ and $WAS_{fw}$), organic C and $\delta^{13}C$ values of aggregates (AG), macroaggregates stable to fast wetting ($MA_{fw}$) and macroaggregates stable to fast wetting and water abrasion ($MA_t$) in the top horizons




| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *TOC g kg⁻¹* | *39.6* | *36.3* | *41.2* | *35.5* | *34.4* | *34.2* | *51.1* | *47.4* | *44.7* | *67.8* | *67.8* | *62.8* |
| WAS % | | 75.6 | 73.0 | | 70.6 | 72.6 | | 65.5 | 71.8 | | 66.8 | 56.5 |

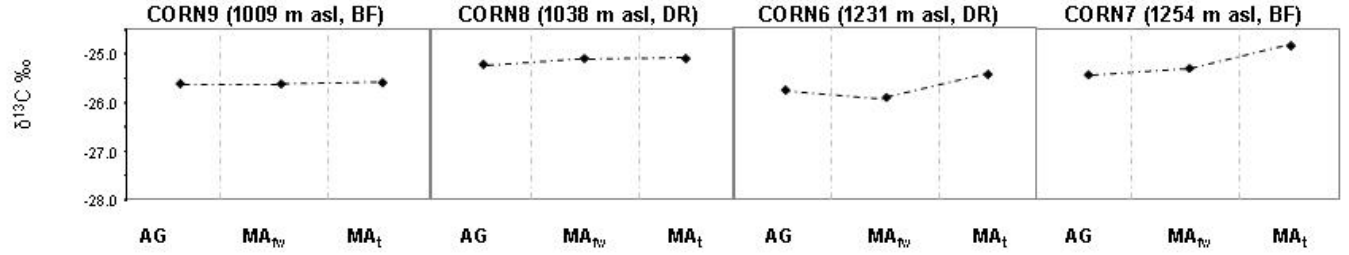



| | | | | | | |
|---|---|---|---|---|---|---|
| *TOC g kg⁻¹* | *47.6* | *50.7* | *48.4* | *61.5* | *62.6* | *51.8* |
| WAS % | | 80.7 | 80.6 | | 54.9 | 50.8 |

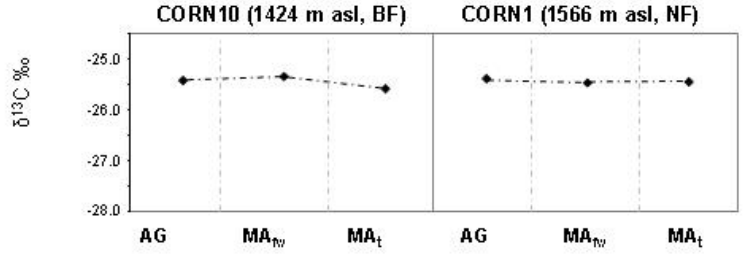



| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *TOC g kg⁻¹* | *91.4* | *94.9* | *124.5* | *73.2* | *74.4* | *76.6* | *40.3* | *39.6* | *39.7* |
| WAS % | | 79.1 | 85.5 | | 66.9 | 77.2 | | 67.0 | 66.4 |

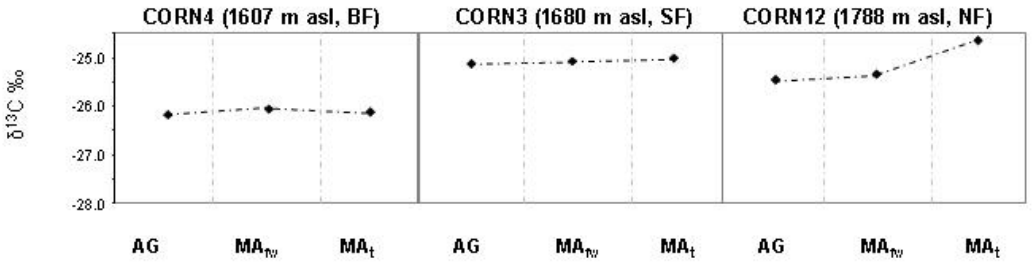
**Figure 3** – Water aggregate stability indexes ($WAS_t$ and $WAS_{fw}$), organic C and $\delta^{13}C$ values of aggregates (AG), macroaggregates stable to fast wetting ($MA_{fw}$) and macroaggregates stable to fast wetting and water abrasion ($MA_t$) in the bottom horizons




| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *TOC g kg⁻¹* | *16.2* | *15.1* | *16.9* | *22.2* | *22.2* | *19.1* | *22.5* | *22.1* | *17.4* | *10.7* | *10.7* | *9.0* |
| WAS % | | 80.9 | 52.1 | | 74.6 | 82.2 | | 68.5 | 65.3 | | 43.3 | 29.9 |




| | | | | | | |
|---|---|---|---|---|---|---|
| *TOC g kg⁻¹* | *24.0* | *25.1* | *25.1* | *15.1* | *17.5* | *14.1* |
| WAS % | | 80.3 | 72.5 | | 67.9 | 65.4 |




| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *TOC g kg⁻¹* | *20.7* | *23.5* | *21.5* | *29.3* | *30.5* | *29.7* | *11.0* | *10.4* | *8.5* |
| WAS % | | 69.6 | 76.7 | | 73.7 | 71.9 | | 75.7 | 89.8 |

**Table 7** – Matrix of correlation between $WAS_t$, $\delta^{13}C$ and enrichment/depletion index in total C, N, Al, Fe, Ca, Mg, K, P and S concentration of macroaggregates stable to fast wetting and water abrasion ($MA_t$) in the top and subsoils

| *top horizons* | $WAS_t$ | $\delta^{13}C$ | TOC | N | Al | Fe | Ca | Mg | K | P | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $WAS_t$ | 1 | | | | | | | | | | |
| $\delta^{13}C$ | 0.204 | 1 | | | | | | | | | |
| TOC | 0.744* | 0.644* | 1 | | | | | | | | |
| N | 0.608 | 0.748* | 0.934** | 1 | | | | | | | |
| Al | -0.442 | 0.149 | -0.342 | -0.239 | 1 | | | | | | |
| Fe | -0.769* | 0.067 | -0.556 | -0.403 | 0.897** | 1 | | | | | |
| Ca | 0.312 | 0.431 | 0.487 | 0.415 | 0.138 | 0.051 | 1 | | | | |
| Mg | -0.419 | 0.071 | -0.317 | -0.171 | 0.837** | 0.824** | 0.251 | 1 | | | |
| K | -0.349 | 0.038 | -0.376 | -0.264 | 0.901** | 0.804** | 0.125 | 0.770* | 1 | | |
| P | 0.218 | 0.693* | 0.573 | -0.264 | 0.473 | 0.288 | 0.729* | 0.533 | 0.350 | 1 | |
| S | 0.615 | 0.650 | 0.799** | 0.757* | 0.154 | -0.141 | 0.583 | 0.239 | 0.088 | 0.876** | 1 |
| *bottom horizons* | $WAS_t$ | $\delta^{13}C$ | TOC | N | Al | Fe | Ca | Mg | K | P | S |
| $WAS_t$ | 1 | | | | | | | | | | |
| $\delta^{13}C$ | 0.005 | 1 | | | | | | | | | |
| TOC | -0.052 | 0.759* | 1 | | | | | | | | |
| N | 0.185 | 0.885** | 0.894** | 1 | | | | | | | |
| Al | 0.076 | 0.096 | 0.088 | 0.134 | 1 | | | | | | |
| Fe | 0.276 | 0.103 | 0.118 | 0.078 | -0.405 | 1 | | | | | |
| Ca | 0.687* | 0.716* | 0.497 | 0.741* | 0.106 | 0.340 | 1 | | | | |
| Mg | -0.475 | -0.427 | -0.129 | -0.438 | 0.156 | -0.23 | -0.658 | 1 | | | |
| K | 0.353 | 0.277 | 0.128 | 0.338 | 0.878** | -0.252 | 0.445 | -0.303 | 1 | | |
| P | 0.416 | 0.620 | 0.748* | 0.732* | 0.288 | 0.477 | 0.746* | -0.086 | 0.375 | 1 | |
| S | 0.386 | 0.714* | 0.841** | 0.869** | 0.376 | 0.074 | 0.749* | -0.213 | 0.458 | 0.885* | 1 |

**$p<0.01$; *$p<0.05$

## 4. Conclusions

Our data showed that the capacity of soil to preserve C was a complex response to the environment condition, as stated by Conant et al. (2011) for soil OM decomposition. In fact, even if SOM confirmed its role as aggregating agents, we obtained different results for stable aggregates in relation to site elevation, vegetation cover and soil depth.

Independently from phytoclimatic conditions, SOM properties suggested that in the aggregates (AG) from subsoil horizons organic materials were both more decomposed and transformed by microbial process and more preserved by metal complexation or interaction with minerals than in top horizons. However, segregation of differently transformed SOM fraction occurred in stable aggregates (MA) according to phytoclimatic condition: in topsoils of beech forest and non-forest site, the worsening of pedoclimatic condition along elevation should inhibit microbial activity, and stable macroaggregates could form around particulate organic matter partially decomposed; in subsoils stable aggregates could form by similar aggregation processes than that of topsoils but only in the case of beech forest, while in non-forest site it seemed that the more intense soil weathering coulpled to SOM quality in vaccinetum enhaced interaction with mineral phases and, consequently, a greater formation of stable aggregates. However, the different aggregation pathways were not related to either aggregate stability or C preservation, and indepently from phytoclimatic condition in topsoil the aggregate resistance to both fast wetting and water abrasion enhanced the preservation of SOM enriched in $^{13}C$. In subsoil, the preservation of C was due to other mechanisms, likely stabilisation of SOM by mineral interactions.

Finally, the use of $^{13}C$ natural abundance approach seems to be a very promising approach to investigate soil C pathways by SOM segregation in stable aggregates, and in future research it could provide new insights into the complexity of soil biophysical process.

## Chapter V: General conclusions

The quantification and qualitative interpretation of the soil aggregates stability indexes in relationship with losses of soil structure cementing agents or soil plants nutrients are of great importance to determine the physical behaviors of soil and to propose the best ways for the soil sustainable management.

The proposed combined method (i.e., determination of aggregate stability and loss of nutrients) based on physical behaviour of aggregates seems to be useful in the mechanistically understanding of the soil C and nutrients fate related to erosion. In agricultural soils (Chapter 2) it allows to identify that the severity of aggregate breakdown affected the quantity of nutrients lost more than their availability. Moreover, it allowed to show that the specific aggregate breakdown mechanisms affected the loss of fertility in different ways: P, K and Mg were the most susceptible elements to water abrasion, while C and N were mainly susceptible to wetting.

By applying a combination of chemical characterisation and aggregate physical fraction, it was possible to determine the effect of SOM properties on aggregate stability and the capacity of soil aggregate to limit SOC, or other elements, depletion (Chapters 3 and 4).

In the studied Chestnut-Douglas fir biosequence (Chapter 3), the content and chemical composition of aggregate associated-organic C were influence by plant species. The chemical composition and origin of SOC has a pronounced effect on aggregates properties and data suggested that broad-leaf trees could give more favourable condition for aggregate associated-organic carbon formation than needle-leaf trees. The different chemical properties of aggregate associated-organic matter also seemed to affect the relative importance of SOM direct (i.e., SOM directly acted as organ cement) and indirect (i.e., SOM affected aggregate stability as organic-metallic cement) mechanisms involved in the aggregate stability and the loss nutrients. In particular, under Douglas fir plants, high presence of carboxylate groups enhanced interactions with metal (Al and Fe) and stabilised aggregates against water abrasion; whereas under Chestnut trees, organic matter direct actions prevalently occurred and fresh, more C-rich organic materials were preserved. Furthermore, under Chestnut trees lower losses of C occurred from water stable macroaggregates than under Douglas fir, suggesting that in our study case SOM direct mechanism was more efficient in C stabilisation in aggregate that indirect mechanism.

The $^{13}C$ natural abundance approach was suitable to study C pathways by SOM segregation in stable aggregates from soils of different phtytoclimatic ecosystems (Chapter 4). Our data showed that the capacity of soil to preserve C was a complex response to the environment condition. In fact, even if SOM confirmed its role as aggregating agents, we obtained different results for stable aggregates in relation to site elevation, vegetation cover and soil depth.

Segregation of differently transformed SOM fraction occurred in water stable aggregates according to phytoclimatic condition, and stable macroaggregates can form both around particulate organic matter partially decomposed and by interaction between organic and mineral phases. However, the different aggregation pathways were not related to either aggregate stability or C preservation, and independently from phytoclimatic condition in topsoils the aggregate resistance to both fast wetting and water abrasion enhanced preservation of SOM enriched in $^{13}C$. In subsoil, the preservation of C was due to other mechanisms, likely stabilisation of SOM by mineral interactions.

Finally, the use chemical characterisation and aggregate physical fraction approach seems to be very promising tool to investigate soil C pathways by SOM segregation in stable aggregates, and in future research it could provide new insights into the complexity of soil biophysical processes.

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